DEMOGRAPHIC AND GENETIC CONSEQUENCES OF SMALL POPULATION SIZE IN REMNANT POPULATIONS OF ARABIS GEORGIANA HARPER (GEORGIA ROCKCRESS)

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THESIS ABSTRACT

The goal of this thesis is to provide a greater scientific understanding of the demographic and genetic consequences of small population size in remnant populations of *Arabis georgiana* Harper (Georgia Rockcress). Specifically, 1) traits associated with phenological progression, fitness and reproductive success were compared between naturally occurring "native" plants and plants grown *ex situ* and subsequently restored to one of the largest remaining populations of *A. georgiana*, and 2) a preliminary analysis of population genetic structure in remnant populations across the species' range was performed. In addition to updating census information on remnant populations of *A. georgiana*, chapter one represents the first critical evaluation of traits associated with the phenological and reproductive success in this species. Moreover, this chapter explores the possible negative effects of *ex situ* restoration practices within natural populations. Although results indicate no significant delays in phenological progression (the timing of flowering and fruit dehiscence), traits associated with fitness and reproductive success (plant size, fruit production, and seed output) were significantly lower in restored plots compared to their native cohorts. In restored plots, plants were 9.3% shorter, produced 44.0% less fruit, had 6.5% fewer fruit dehisce, produced 14.4% fewer seeds, and had a 13.0% reduction in seed weight compared to native plants. These results suggest that genetic bottlenecks potentially invoked through *ex situ* conservation efforts can have a negative impact on the restoration of remnant populations. The second chapter of this thesis includes an updated population census of the species as well as the first confirmation of its genetic identity. To evaluate the magnitude of genetic structuring across the range of *A. georgiana,* potential variation in ploidy, cpDNA haplotypes and

microsatellite markers was also evaluated. Census data revealed no species-wide pattern for population growth or decline compared to data collected in 2005. The *rbcL* barcode generated for this species were confirmed as a unique haplotype when compared with other co-occurring members of Brassicaceae. Analysis of genomic DNA content using flow cytometry showed no variation in ploidy across the species range and suggests that *A. georgiana* is most likely octoploid; however, visual confirmation of chromosome number is still required. No sequence variation was found among *trnL* (UAA) intron cpDNA haplotypes. Of the seven microsatellite loci screened for this study, one locus (DnB220) revealed significant genetic structuring among 101 samples across 10 populations. Three genetic clusters (K=3) were found, each population having a common and private allele, with 11% of all individuals sampled being homozygous for the common allele. Collectively, this research program will contribute to the effective management and conservation of a narrow endemic whose fragmented populations are steadily declining.

CHAPTER 1

Assessing the effect of restoration on phenological progression and fitness of rare *Arabis georgiana* Harper (Georgia Rockcress)

Abstract

Understanding the genetic and demographic impacts of plant restoration efforts can help land managers more efficiently navigate challenges associated with the conservation of rare taxa. More specifically, the provenance and quality of plants used in replanting programs can impact the phenological progression, fitness and reproductive success of restored populations. In this study, I evaluated the effects of restoration in rare populations of *Arabis georgiana* Harper (Georgia rockcress), a short-lived perennial endemic to Georgia and Alabama. *Arabis georgiana* exists in 17 populations that range in size from 12 to greater than 2000 plants. To date, several attempts have been made to augment remaining populations with plants grown *ex situ* from seeds collected at home sites. To evaluate the efficacy of these restoration efforts, I measured a series of phenological, fitness, and reproductive traits between three native and three restored plots within one of the largest known populations of *A. georgiana.* Although results indicate no significant delays in phenological progression (the timing of flowering and fruit dehiscence), traits associated with fitness and reproductive success (plant size, fruit production, and seed output) were significantly lower in restored plots compared to their native cohorts. In restored plots, plants were 9.3% shorter, produced 44.0% less fruit, had 6.5% fewer fruit dehisce, produced 14.4% fewer seeds, and had a 13.0% reduction in seed weight compared to native plants. Collectively, these results suggest that genetic

bottlenecks potentially invoked through *ex situ* conservation efforts can have a negative impact on the restoration of remnant populations. This study provides a valuable first assessment of the reproductive biology of *A. georgiana* and contributes directly to the conservation of this rare species.

INTRODUCTION

Dramatic declines in population size can cause a population to go through a genetic bottleneck that can ultimately lead to a reduction in genetic diversity (Ellstrand & Elam, 1993; Wiegand *et al.,* 1998; Lu *et al.,* 2005). Subsequent mating between closely related individuals can lead to reductions in fitness due to inbreeding depression (Burskirk & Willi, 2006; Fredrickson *et al.,* 2007; Charlesworth & Willis, 2009). Individuals suffering from inbreeding depression have been shown to exhibit decreased fitness in response to environmental stress (Paschke *et al.,* 2003; Fredrickson *et al.,* 2007; Waller *et al.,* 2008). For rare taxa, a decline in fitness can ultimately create an "extinction vortex" for remaining populations (Newman & Pilson, 1997; Keâry *et al.,* 2000). Although, the impact of genetic bottlenecks on rare animal taxa has been well described (see reviews in Ebenhard, 2000; Hendrick & Kalinowski, 2000) relatively few studies fully explore its ramifications in rare plant populations.

For plants, the impact of genetic bottlenecking can be especially profound. For example, inbreeding depression may not only manifest in reductions in fitness (Galloway *et al.,* 2003; Waller *et al.,* 2008) but also result in delays in phenological progression for flowering (Galloway & Burgess, 2009; Munguı´a-Rosas *et al.,* 2011) and fruiting (Galloway & Burgess, 2009; Anderson *et al.,* 2011). Furthermore, negative impacts associated with genetic bottlenecking may result in differential rates of extinction for rare plant taxa depending on a taxon's particular life history (Ellstrand & Elam, 1993; Angeloni *et al.,* 2011). For example, delays in flowering have been shown to lower the frequency of annuals in natural populations of *Campanulastrum americanum* (American bellflower) (Galloway & Burgess, 2012). Should such a delay occur in rare plant

populations, it seems likely that reduced reproductive output (and hence fitness) may further contribute to the decline of a species. Although, the role of genetic bottlenecking in rare plant populations has been discussed (Schmeske *et al.,* 1994; Cole, 2003; Oleas *et al.,* 2012), its role in restoration has rarely been tested. Specifically, how delays in phenology and reductions in fitness manifest in restoration efforts remains unknown.

Increasingly, *ex situ* restoration programs have been used to re-plant or augment rare plant populations. When invoked, *ex situ* populations are generally established by collecting seeds and/or cuttings from natural source populations of a given target species (Engelmann & Engels, 2002). During collection, however, the total genetic variation that exists within a particular population is rarely sampled resulting in an *ex situ* population that has gone through a genetic bottleneck (Brown, 1992; Husband & Campbell 2004; Volis & Blecher 2010). For example, reduced genetic variation after eighteen years of *ex situ* conservation has been demonstrated in *Cochlearia polonica* Brassicacea (Rucińska & Pulchalski, 2011) when compared to the source populations. Although such reductions in diversity may be expected in restoration efforts that involve *ex situ* plant collections (Williams, 2001; Husband & Campbell 2004; Volis & Blecher 2010), direct phenological and fitness comparisons between plants that have gone through *ex situ* genetic bottlenecks and those that exist in native populations are lacking.

One plant species that may be suffering from genetic bottlenecking as a result of *ex situ* restoration efforts is *A. georgiana* (Brassicaceae). *Arabis georgiana* is morphologically distinct from other members of the genus occurring in the southeastern United States in having petals and siliques 6-9mm and 5-7 mm in length, respectively (Harper, 1903; Patrick *et al.,* 1995) (Fig. 1). This species is not only distinct but is also a

rare, narrow endemic found on eroding river-banks in Georgia and Alabama (Chafin, 2007). Remnant populations are known on only seven river systems with populations on the Coosa and Chattahoochee rivers spanning both states (Moffett, 2007; Schotz, 2010) (Fig. 2). Due to its limited range, *A. georgiana* is listed as threatened in Georgia and is a federal candidate species for listing under the Endangered Species Act. It was originally assessed as a species of concern by the USFWS in 1993, and census information indicates that this species has declined in numbers since that time (Moffett, 2007: Norquist, 2009; Schotz, 2010). This species is imperiled primarily due to habitat loss and degradation (Moffett, 2007; Norquist, 2009; Schotz, 2010). Furthermore, there is interest in the conservation of this species due to its unique status as the only true member of the genus *Arabis* found within Alabama or central and southern Georgia (Al-Shehbaz, 2003; Koch *et al.,* 2010; Weakley, 2012).

Recent restoration efforts of *A. georgiana* populations include *ex situ* propagation and subsequent augmentation of the largest known population of the species, which occurs in Harris County, Georgia (Georgia Plant Conservation Alliance, 2010). In 1992, an unknown quantity of seed was collected from this population and propagated for future restoration. Seed collected from the home site was planted in an *ex situ* garden and allowed to randomly mate and self-seed for thirteen years. In 2006, a subsample of seed from this *ex situ* garden population was collected for *ex situ* propagation and subsequent return to the home site. (Henning von Schmeling, *personal communication* 2011). In 2008, 103 two year-old plants were planted into plots at the home site for future monitoring, here after referred to as restored plots. Native monitoring plots at the home site, containing pre-existing naturally occurring plants were established for long-term

monitoring in 2009. While both restored and native plots contain *A. georgiana* derived from the Harris County site, the reproductive histories of these two groups are very different. The degree to which these differences manifest into substantial impacts on phenology and fitness due to *ex situ* genetic bottlenecking remains unknown.

To evaluate the efficacy of these restoration efforts, a series of phenological traits as well as traits associated with fitness were compared between restored and native plots. Because restored plants have effectively experienced a genetic bottleneck through the process of *ex situ* propagation, I predicted that these plants will exhibit delays in phenological progression and reduced fitness compared to plants growing naturally in native plots. Understanding the impact of this *ex situ* restoration program may help land managers more efficiently navigate conservation challenges for *A. georgiana* as well as other rare taxa.

METHODS

Study Site and plot design

Restored and native plots were established in 2008 and 2009, respectively (Table 1). These plots were located in one of the largest known populations (~ 1500 plants in 2011) of *A. georgiana,* which occurs on gneiss outcrops along the Chattahoochee River in Harris County, Georgia. Restored and native plots were located in close proximity to each other (less than 30 meters) and distributed evenly across the site which spans 300 meters (Fig. 3). Restored and native plots were characterized for the following nine soil variables: pH, calcium, potassium, magnesium, manganese, available nitrate (NO_3^-), phosphorus and zinc. Soil samples were collected within 1 m of each plot at adjacent

ordinal positions (east and west). Leaf litter was removed from the soil surface and a 12 cm deep x 10 cm wide sample was taken. All samples were sent to the University of Georgia Soil, Plant and Water Analysis lab for analysis. All values other than pH were analyzed in grams per m^2. Mean values for each plot were then calculated and compared among treatments using a one-way ANOVA. To meet the assumptions of homoscedasticity for the ANOVA model, values for magnesium and manganese were log-transformed.

Phenological and fitness components

To determine if restored versus native plots exhibited reduced fitness as a result of genetic bottlenecking, each plot was visited weekly during the 2010 reproductive season (March-October). Here, a number of phenological (average flowering day and average date of first dehiscence) and fitness (plant height at first flower, total number of fruit produced, proportion of fruit dehisced, seed number and seed weight) traits were measured. Specifically, average flowering date (AFD) was calculated using the formula given by Nuismer & Cunningham (2005) as: AFD = $\sum_{i-1}^{N}(n_i x_i | \sum_{i-1}^{N} ni)$ where n is the number of flowers open on an individual plant on day *i,* x_i is the Julian date of day *i* and N is the last day the plant had an open flower. Average date of first dehiscence (ADFD) is a calculated in the same manner as AFD but Julian date is calculated from the first census date flowering was observed and N is the day on which final dehiscence for all fruits occurred.

Measures of fitness include plant height at first flower, the total number of fruit produced per plant, proportion of fruit dehisced, seed number per fruit and total seed weight per plant. Plant height at first flower was assessed by measuring plant height on the first date each respective plant was observed to flower. The total number of fruit per plant was recorded after flowering had ceased and mature fruit pods were clearly apparent. The proportion of dehisced fruit was calculated as the total number of fruit in dehiscence divided by the total number of fruit observed to be developing after cessation of flowering: fruit that aborted or failed to open prior to final desiccation of the reproductive stalk were recorded as indehiscent. Average number of seeds per fruit was assessed by gathering two mature fruits judged as being average sized for a particular plant. Fruits were collected prior to dehiscence and the total number of viable seeds per fruit was counted. After counting, all seeds contained within each fruit were collectively weighed and mean seed weight per fruit across two fruits was calculated.

All response variables were compared among restored and native plots using a one-way analysis of variance (ANOVA) (JMP statistical software Version 10.0, SAS Institute 2012). ANOVA was also used to compare differences among plots within each plot type with a Tukey's HSD. To meet the assumptions of normality, values for AFD, ADFD, total fruit per plant, and total seed weight per plant were log-transformed while ArcSine-squareroot transformations were performed on the proportion of dehisced fruit.

RESULTS

The following eight soil attributes were evaluated for differences between plot types: pH, calcium, potassium, magnesium, manganese, available nitrate (NO_3^-),

phosphorus and zinc. None of the soil attributes evaluated in this study varied significantly among treatments mean (Table 2). Although there were no significant differences between restored and augmented plot types, between plot means for pH ranged from 5.38 to 6.47, calcium varied from 164.15 to 342.81 g/m^2, potassium ranged from 34.1 to 74.6 g/m^2, manganese ranged from 3.9 to 9.0 g/m^2, magnesium ranged from17.8 to 53.2 g/m^2. Available Nitrate ranged from 0.5 to 4.4 g/m^2 and zinc ranged from 0.4 to 1.5 g/m^2.

Traits associated with phenological progression (Average Flower Date [AFD] and Average Date of First Dehiscence [ADFD]) were not significantly different between plot types (AFD $F_{1,76} = 0.09$, $p > 0.05$; ADFD $F_{1,76} = 0.004$, $p > 0.05$) in either case (Fig. 4), with both restored and native plots producing flowers on census day 12. On average restored and native plots both dehisced fruits on census day 122. Significant differences between plots were found for AFD which ranged from census day 9 to census day 15.3 ($F_{5,72} = 7.57$, $p < 0.0005$) but not for ADFD which ranged from census day 105 to census day 130 ($F_{5,72} = 1.72$, $p > 0.05$) (Fig. 5). Differences between individual plots for AFD was due to significant delays in flowering for restored plot C (9.6 days) and native plot 2 (9.0 days) compared to the overall plot mean of 12.3 days.

Traits associated with fitness (plant height at first flower), were significantly lower in plots comprised of restored plants compared to their native cohorts. Restored plants were significantly shorter (mean = 41.3 cm) than native plants (mean = 45.5 cm) ($F_{1,76} = 5.05$, $p < 0.05$) (Fig. 6). There were no significant differences between individual plots for height at first flower ($F_{5,72} = 1.72$, $p > 0.05$) (Fig. 7).

The mean difference in fruit production between restored and native plants was significantly different between groups with restored plants producing an average of 19 fewer fruits per plant (mean = 24.3) than native plants (mean = 43.5) ($F_{1,76} = 10.93$, $p < 0.01$) (Fig. 8a). Fruit production was varied significantly between individual plots (range of 13.1 to 58.1; $F_{5,72} = 4.66$, $p < 0.005$) however, plots did not vary within treatments (Fig. 9a). The proportion of fruit dehisced in restored plots (mean = 80.7%) was 6.5% lower in than native plots (mean = 86.3%) (Fig. 8c). Although observed dehiscence was lower in restored plots, this trait was not significant between plot types ($F_{1,76} = 2.69$, $p > 0.05$) or individual plots ($F_{5,72} = 1.58$, $p > 0.05$) (Fig. 8 & 9).

Restored plots on average produced 5 fewer seeds per fruit (mean = 30.0) than plots of native plants (mean = 35.0) ($F_{1,76} = 17.64$, $p < 0.0001$) (Fig. 8b). Additionally, there was a significant reduction of 13.0% in the overall mean weight of aggregate seeds collected from two fruits per plant in restored plots (mean = 8.8 mg) compared to native plots (mean = 10.2 mg) ($F_{1,75} = 3.89$, $p < 0.05$) (Fig. 8d). Post hoc analysis for seed number revealed significant differences in native plots 2 and 3 from plots 1, A, B and C ($F_{1,72} = 11.94$, $p < 0.0001$) (Fig. 9b). Native plots 2 and 3 were also significantly different from plots 1, A, B and C for aggregate seed weight ($F_{1,72} = 11.22$, $p < 0.001$) (Fig. 9d).

DISCUSSION

The study attempts to determine if there is evidence of reduced fitness in restored versus native plots of *A. georgina* attributable to plant provenance. Restored plants were observed to have reduced performance for traits associated with fitness and reproductive success, namely, height at first flower, fruit number, seed number, and seed weight (Table

3). Collectively, these results suggest that plants used for restoration in this study are experiencing some level of inbreeding depression due to *ex situ* genetic bottlenecking.

It is important to consider the differences in environmental conditions that restored versus native plants were subjected to for the time period between seed collection and outplanting of restored plants back to the home site. Given that the *ex situ* site was approximately 100 miles north of the home site, returning plants to the home site where different environmental conditions were present could have negatively impacted performance of the restored plants. It is possible that *ex situ* lineages, grown for several generations under *ex situ* environmental conditions, have experienced local adaptation for traits associated with fitness and reproductive success. More research, such as a reciprocal transplant experiments, may elucidate the relationship between the sources of genetic versus environmental contributions to localized adaptation *ex situ* and differences in performance between restored and native plants in this study.

No significant differences in phenological progression (AFD & ADFD) were observed between plot types in this study. There are several possible explanations for the disparity between the reduced performance of restored plots for traits associated with fitness and reproductive success versus the lack of differences between plot types for those traits associated with phenology. For some plants, such as *Campanulastrum americanum*, the maternal genotype may play a dominant role in determining offspring phenology (Galloway *et al.,* 2009). It is possible that for *A. georgiana*, similar maternal effects may slow the selection response of phenological progression to environmental variation. Here, the localized response to selection for traits associated with phenology *ex situ* may not be a strong as that for traits associated with fitness and reproductive success.

A review of available literature has indicated that *A. georgiana* may be an autotetraploid (Koch *et al.,* 2010; Warwick & Al-Shehbaz, 2006) and thus may further explain the lack of phenological differences found between native and restored plots. Further research investigating the role environment, maternal effects and inbreeding depression on the phenological progression of this species is needed.

Significant reductions in the quantity of fruit and seed produced by restored plants in native habitats may negatively impact existing native plants. If restored plants consume an equivalent quantity of environmental resources, such as space, soil nutrients and available water, but produce lower quality offspring then, environmental resources allocated to restored rather than native plants will yield a lower net reproductive benefit to the native species. The decrease in reproductive output observed in restored plants in this study is mirrored in other studies on the effect of inbreeding depression as a result of management (Volis & Blecher 2010; Young & Pickup, 2010; Ritchie & Krauss, 2012). Similar reductions in plant size and reproductive output have been observed in *Silene* species from small populations with reduced genetic variation (Lauterbach *et al.,* 2011). Based on differences in reproductive history between the native and restored plots of *A. georgiana*, the bottleneck effect seems evident.

Although not evaluated in this study, the negative effects of gene flow from restored plots to native plants also represents an additional potential impact of the persistence of native populations that merits further research. It is important to note here that recruitment occurred within restored plot A after augmentation and prior to this study. *Arabis georgiana* is capable of reproducing within six months of germination and annually thereafter. Because restored plots were established in December 2008,

reproduction in 2009 between restored plants and plants of unknown origin occurred, resulting in observed recruitment in restored plot A and net increase of 18 individuals. It is assumed, based on proximity, that new plants occurring within this restored plot are F1 offspring, with restored individuals serving as the maternal parent. Because native *A. georgiana* plants do occur within the vicinity of the restored plot, native plants could have sired these offspring which creates a unique opportunity for the effects of inbreeding depression on both parent plants and offspring to be studied.

Significant differences between native and restored plots were found even in the presence of recruitment and probable gene flow from native plants into restored plots. This indicates that the effect of inbreeding depression in restored plots appears to be strong. Because maternal genotype is known to play a dominant role on offspring phenotype for some plant species, the full effect of inbreeding depression in newly recruited plants may not be observable until the year following germination (Burgess & Husband, 2004; Burgess *et al.,* 2007; Galloway & Burgess, 2009). Since maternal genetic effects have been shown to impact offspring traits such as rosette size, seed size, and flowering time (Galloway *et al.,* 2009), further transgenerational differences between plot types in *A. georgiana* represents a potential increase in the magnitude of inbreeding depression compared to the levels detected in this study.

While none of the eight soil attributes differed significantly between plot types, additional environmental variables such as light and temperature could be a significant source of variation in the response variables measured in this study. Although not measured during this time of this study, HOBO Pendant® Temperature/Light Data Loggers were used to measure the extent of light and temperature variation between plot

types in the following year from November 2010 until November 2011. Previous reports on this species indicated that *A. georgiana* does not tolerate intense shading and prefers habitats with higher light (Moffett, 2007). Therefore restored plants were intentionally planted in higher light zones prior to commencement of this study. Interestingly, regardless of significant variation in total light received per day (lum/ft^2), restored plots are not significantly different from native plots for mean daily temperature ($F_{1,46} = 4.62$, $p > 0.05$) (Appendix A). Mean temperature was also not significantly different between plots ($F_{3,44} = 0.05$, $p > 0.05$) (Appendix A). The lack of environmental variability between plot types strengthens the argument that inbreeding depression may have contributed to the reduction in performance within restored plants for several of the traits measured.

Recommendations for management

Habitat degradation, selection pressures from invasive species and climate change are accelerating the rate at which rare taxa are becoming extinct (Ellstrand & Elam, 1993). The primary purpose of this study was to evaluate the efficacy of restoration efforts that focus on increasing population size in the absence of data on the genetic diversity of plants used for restoration. This study shows that this strategy may not have the maximum possible impact in facilitating the persistence of a rare species. During future collection of *A. georgiana* seeds for restoration efforts, records of both collection site and methodology should be recorded. Steps to ensure that optimal genetic diversity is both collected and maintained within *ex situ* populations should also be taken. To prevent localized adaptation to climactic conditions distinct from those encountered at home sites, yearly collection of seed from home sites could be performed. Alignment of *ex situ*

propagation sites to geographic locations of *A. georgiana* has already been undertaken and may serve to mitigate potential *ex situ* adaptation. Reoccurring seed collection must be performed cautiously to prevent depletion of the seed bank at home sites. Given the robust size and stability of populations such as the one in Harris County where this study was conducted, restoration efforts might be best optimized by focusing on smaller and more critically imperiled populations of this species.

In addition to serving as an evaluation to inform management for *ex situ* safeguarding efforts, this study allows some inferences to be drawn about the impacts of reduced population size on performance of *A. georgiana* for the traits measured within this study. The *ex situ* population evaluated during this study has in effect been subjected to a population bottleneck. The offspring produced as a result of this bottleneck had reduced performance for several of the traits evaluated. Field observations made by land managers have noted similar reductions in vigor (Georgia Plant Conservation Alliance, 2010) for this species within small isolated populations. The results of this study indicate that further evaluation of the genetic structure of this species is needed in order to fully understand the impact of small population size, which contributes to inbreeding depression in *A. georgiana*. Overall the results of this study provide a valuable assessment of the biology of this rare plant species and contribute to the future management of remaining *A. georgiana* populations where genetic bottlenecks due to small population size and *ex situ* restoration efforts may negatively impact persistence.

CHAPTER 2

Assessing the effect of habitat fragmentation on the genetic structure of remnant populations of *Arabis georgiana* Harper (Georgia Rockcress)

ABSTRACT

Habitat fragmentation can have profound impacts on the genetic viability of many rare plant species. *Arabis georgiana* Harper (Georgia rockcress) is a rare, short-lived perennial, endemic to eroding riverbanks in Georgia and Alabama. Currently only 17 populations remain, most of which are isolated from one another due to habitat fragmentation. To confirm the genetic identity and level of population structure for this species, a population census was conducted across the range and potential variation in ploidy and cpDNA haplotypes was evaluated. Additionally, seven microsatellite loci were screened for nuclear variation within the species. Census data revealed no species-wide pattern for population growth or decline compared to data collected in 2005. The *rbcL* barcode generated for this species were confirmed as a unique haplotype when compared with other co-occurring members of Brassicaceae. Analysis of genomic DNA content using flow cytometry showed no variation in ploidy across the species range and suggests that *A. georgiana* is most likely octoploid; however, visual confirmation of chromosome number is still required. No sequence variation was found among *trnL* (UAA) intron cpDNA haplotypes. Of the seven microsatellite loci screened for this study, one locus (DnB220) revealed significant genetic structuring among 101 samples across 10 populations. Three genetic clusters (K=3) were found, each population having a common and private allele, with 11% of all individuals sampled being homozygous for

the common allele. These results provide a valuable first assessment of the genetic identity and structuring of this species and contribute to the future management of remaining *A. georgiana* populations where genetic drift due to fragmentation may limit evolutionary potential.

INTRODUCTION

Habitat fragmentation can have serious consequences for long-term species viability (Falk & Holsinger, 1991; Schemske *et al.,* 1994), resulting in increased rates of predation and loss of reproductive opportunities (Lande, 1988; Tallmon *et al.,* 2003), both of which can decrease population stability and growth (Templeton *et al.,* 1990; Franklin *et. al,* 2002). As the number of individuals within a species declines due to these impacts, genetic diversity is invariably lost which can increase the probability of extinction (Byers & Waller, 1999; Cruzan, 2001; Buskirk &Willi, 2006; Leimu *et al.,* 2006). For plant species, the consequence of fragmented habitats can be particularly profound because mobility is often limited to the local distribution of seed and/or pollen (Leimu *et al.,* 2006; Marini *et al.,* 2012). Hence, the sessile nature of many plant taxa means that even a small amount of fragmentation can have a profound impact on persistence (Ellstrand & Elam, 1993; Gonzalez-Varo *et al.,* 2009).

The magnitude of the impact from habitat fragmentation on a species can be measured by determining the genetic structure of remnant populations (Newman & Pilson, 1997; Cruzan, 2001; Koch *et al.,* 2003; Dobes *et al.,* 2004). Species with small isolated populations tend to have high population structuring (Cruzan, 2001; Zheng *et al.,* 2012), while in widely distributed species, population structure is low (Ellstrand & Elam, 1993; Parchman *et al.,* 2011). Although some population structuring within a species is desirable and can increase a species ability to adapt (Cruzan, 2001; Prunier *et al.,* 2012), high levels of genetic structuring can indicate the occurrence of habitat fragmentation (Templeton *et al.,* 1990; Cruzan, 2001; Taylor & Keller, 2006) or complex spatial structuring within a population (Ellstrand & Elam, 1993; Parchman *et al.,* 2011).

Furthermore, if a species has a high proportion of small and genetically homogenous populations, that species is at greater risk of suffering from the negative effects of both genetic drift and inbreeding depression (Newman & Pilson, 1997; Burskirk & Willi, 2006), which may lead to an increased likelihood of extinction (Ellstrand & Elam, 1993; Schemske *et al.,* 1994; Honnay & Hans Jacquemyn, 2007). Ultimately, the identification of genetically depauperate populations for restoration efforts as well as the conservation of those that are genetically distinct can increase the chances of long-term survivorship of a species (Schemske *et al.,* 1994; Newman & Pilson, 1997; McKay, 2001; Fallon, 2005).

Determining current levels of species abundance and distribution through a population census is an important process in assessing the viability of a species of conservation interest (Lande, 1988; Thomas *et al.,* 2011). Data on current population size is essential when evaluating potential risk of extinction due to both stochastic and deterministic threats (Schemske *et al.,* 1994; Paschke *et al.,* 2003; Frye, 2005). Data collected during a population census is also used to assess conservation listing status while the census itself may provide an opportunity for collection of sample material for future genetic analysis of a population of interest (IUCN, 2001; Garcia-Barriuso *et al.*, 2012).

In addition to establishing species census population size, testing species identity is vital to conservation (Gonzalez-Varo *et al.,* 2009; Burgess, *et al.,* 2011). The use of certain plastid regions identified as DNA barcodes, has proven to be a highly effective tool for establishing sample identity (Armstrong & Ball, 2005; CBOL Plant Working Group: Hollingsworth *et al.,* 2009) and is feasible even when traditional identification methods are not possible due to lack of identifying structures or the presence of

cryptospecies (Herbert *et al.,* 2004; Lahaye *et al.,* 2008 ; Kesanakurti *et al.,* 2011). For land plants, the *rbcL* coding region, has been identified as one part of the two-locus barcode for land plants adopted by the Consortium for the Barcode of Life (Fazekas *et al.,* 2008; CBOL Plant Working Group: Hollingsworth *et al.,* 2009; Kesanakurti *et al.,* 2011). When used as a single locus barcode, this region has been shown to routinely produce high quality bidirectional sequences (Fazekas *et al.,* 2008; Burgess *et al.,* 2011) suitable for species identity resolution in approximately 61% of sequences sampled (CBOL Plant Working Group: Hollingsworth *et al.,* 2009). Establishing and using DNA barcodes can aid conservation and research by facilitating correct species identification, recognition of the taxonomic rarity and appropriate levels of conservation planning (Blaxter *et al.,* 2005; Francis *et al.,* 2010; Burgess *et al.,* 2011).

Once species identity has been established, analysis of both population structure and within species diversity is important. A comprehensive understanding of potential genetic and demographic impacts of habitat fragmentation can aid conservation planning (Schemske *et al.,* 1994; Paschke *et al.,* 2003; Frye, 2005). Species complexes, which are common among plants, can complicate this analysis (Mummenhoff *et al., 2*001; Cires & Prieto, 2012). Historically, many methods have been employed to estimate population-level variation. Estimates of ploidy have been useful for species that have been subjected to habitat fragmentation (Dart *et al.,* 2004; Dobes *et al.,* 2006) in order to determine current intraspecific relationships. The use of certain variable cpDNA regions in evaluating species expansion and subsequent fragmentation has also been used. The chloroplast *trnL* (UAA) intron has been shown to be useful for examining relationships within species complexes, evaluating species identity, and determine phylogenetic

relationships between long separated populations (Sharbel & Mitchell-Olds, 2001; Dobes *et al*., 2004; Taberlet *et al.,* 2007; Scarcelli *et al.,* 2011). These techniques combined with an assessment of nuclear variation using microsatellite markers, have proven valuable to fully evaluate the genetic identity of species subjected to intense habitat fragmentation (Goldstein *et al.,* 1999; Chambers & MacAvoy, 2000; Skrede *et al.,* 2009; Parchman *et al.,* 2011).

A plant species that is suspected to be suffering from habitat fragmentation is *A. georgiana* Harper (Georgia rockcress). *Arabis georgiana* is a narrow endemic herb with remaining populations in Georgia and Alabama, USA (Patrick *et al.,* 2005, Chafin, 2007). This species is known to occur in five different geologic regions with 17 known population sites (Moffett, 2007; Schotz, 2010) (Fig. 2). These geologic regions are characterized by dramatically different types of soil and bedrock, which may impact the pH and soil nutrients in which *A. georgiana* grows (Montgomery, 2008). This rare species is listed both globally and at the subnational level as critically imperiled (G1/S1) (NatureServe, 2012). Furthermore it is also listed as threatened within the state of Georgia and is a candidate for federal listing (Norquist, 2009). Destruction of vegetative buffer along stream banks, logging, development, and quarrying have fragmented populations and reduced both habitat size and quality (Moffett, 2007; Schotz, 2010). Reductions in the size of some known populations of *A. georgiana* within Georgia have been reported since the last formal census conducted in 2005 (Georgia Plant Conservation Alliance, 2010), however up-to-date census information for all populations is required for a thorough evaluation of putative declines.

Recent re-classification of the genus *Arabis (*Al-Shehbaz 2003) also suggests that confirmation of the genetic identity of remaining *A. georgiana* may be necessary to confirm current census estimates and putative declines due to habitat fragmentation. This is due in large part to the fact that this species is sympatric with *Boechera laevigata* which was previously classified as *A. laevigata* and easily confused with *A. georgiana* due to morphological similarity (Moffett, 2007; Chafin, 2007; Norquist, 2009). To confirm *A. georgiana* as a distinct species from *B. laevigata,* the *rbcL* DNA barcoding region of the chloroplast genome may be useful to confirm the genetic identity of remaining *A. georgiana* (Fazekas *et al.,* 2008; Burgess *et al.,* 2011).

A number of molecular techniques show promise for assessing genetic variation within remaining *A. georgiana* populations. Firstly, many species within the Brassicaceae are known to have variable chromosome size or shape between populations (Dart *et al.,* 2004; Grundt *et al.,* 2005; Warwick, & Al-Shehbaz. 2006). Because *A. georgiana,* in particular, belongs to a clade of North American *Arabis* known to be diploid, tetraploid or octoploid (Koch, *et al.,* 2010) knowledge of chromosomal level variation may also be useful for understanding *A. georgiana* population structure. Secondly, variation within the *trnL* (UAA) intron is known within some species of the Brassicaceae (Sharbel & Mitchell-Olds, 2001, Karl *et al.,* 2012) and has been used to determine the phylogeny of long separated populations (Mummenhoff *et al.,* 2001, Dobes *et al.,* 2004). If populations of *A. georgiana* have been fragmented for a sufficient period of time, variation in the *trnL* (UAA) intron sequence may also facilitate an assessment of current population genetic structure in remaining populations. Finally, population structure in *A. georgiana* can be evaluated through the use of microsatellites (Chambers & MacAvoy, 2000; Hauser *et al.,*

2002). Designing species specific microsatellites is time consuming, expensive, and requires specialized equipment. In a paper written by Skrede *et al.* (2009), 65 microsatellite loci were evaluated for cross genus transfer within the Brassicaceae and found to have a 6-18% success rate.

The goal of this research is to determine the genetic structure of remaining *A. georgiana* populations within Georgia and Alabama. To address this goal the following objectives were be addressed: 1) conduct a population survey to estimate the current number of individuals within each remaining population 2) confirm the genetic identity of this species using the *rbcL* barcode 3) assess the genetic structure of remaining populations by quantifying variation in a) DNA content; b) the *trnL* (UAA) intron of the chloroplast genome; and c) microsatellite loci. Collectively, the data obtained through these three lines of investigation can answer questions about how population size influences the level of genetic variation within this species (Chambers & MacAvoy, 2000; Kikuch & Isagi, 2002), arming land managers with information that may influence management actions and conservation priorities (Ellstrand & Elam, 1993; Fallon, 2005).

METHODS

Population census and tissue sampling

Between March 2010 and August 2011 the number of individuals per population in Georgia was determined by direct counts. Direct counts for all Alabama populations were performed during 2009 and 2010 (Schotz, 2010; The Alabama Natural Heritage Program, 2011). Historical data on population size for six Georgia populations and one Alabama population was available from a Georgia Department of Natural Resources

from a 2005 survey (Moffett, 2007). Georgia populations for which census data was available included Black's Bluff, Whitmore's Bluff, Oostanaula Bluffs, Goat Rock, Fort Benning and Fort Gaines. The one Alabama population census for which 2005 census data was available was the Alabama population of *A. georgiana* on Fort Benning. Historical data on population size were compared to current census data using a Chi-Square goodness-of-fit test to determine if changes in population size were significantly different between updated and historical census data.

While performing census counts, leaf tissue was collected from ten out of the seventeen total populations including six Georgia and four Alabama, respectively (Fig. 10). Leaf samples were collected from both fertile and vegetative plants. Samples were taken from at least 10% of the individuals within a population of 100 or more plant while all individuals from small populations were sampled so that a minimum sample size from any given population was at least 12. Approximately five square centimeters of leaf tissue was collected from each plant in order to obtain enough material for multiple DNA extractions. Collected tissue was stored in coin envelopes and dried in silica gel according to methods used by Burgess *et al.*, 2011.

Confirmation of genetic identity

Confirmation of the genetic identity of *A. georgiana* was performed on a subset of the individuals (N=7) that were collected across four of the six Georgia populations. This subset contained samples from two of the three populations where *B. laevigata* co-occurs with *A. georgiana*. In addition, two samples of *B. laevigata* from sympatric populations were included (Table 4).

To prepare samples for DNA extraction, ~ 20mg of silica-dried leaf material was pulverized for 40 seconds at 6.0 m/s using FastPrep tissue-disrupter (MP Biomedicals, Solon, OH, USA). Following incubation in lysis solution for 15 minutes, total genomic DNA was extracted from each sample using MP Bio FastDNA spin kits (MP Biomedicals, Solon, OH, USA). After DNA extraction, the *rbcL* gene region of the chloroplast genome was amplified using forward primer *rbcL*aF and reverse primer *rbcL*ajf 634R (Table 5). Reagents were combined in 20.06µl of reaction mixture containing 0.16µL of 5U/µl AmpliTaq Gold Taq polymerase (Applied Biosystems, Branchburg, New Jersey, USA), 10µl of 10% trehalose, 2µl of 10X Amplitaq Gold buffer, 2µl of 25mM $MgCl_2$, 2µl of 2mM premixed dNTP's (Applied Biosystems, Branchburg, New Jersey, USA), 0.2µl of 10µM of each primer, 1.5µl nuclease free dH_2O, and 2µl of template DNA.

The protocol for amplification of the *rbcL* gene region was as follows: initial denaturing for 5 minutes at 95.0 °C; touchdown step of 5 cycles: 1 minute at 95.0 °C, 40 seconds at 58.0 °C - 54.0 °C, 1 minute at 72.0 °C; 30 cycles of 1 minute at 95.0 °C, 40 seconds at 54.0 °C and 1 minute at 72.0 °C; followed by a 5 minute final extension at 72.0 °C and final hold at 4.0 °C. Unpurified forward and reverse PCR products were shipped on ice to Functional Biosciences Inc. (Madison, Wisconsin, USA) for sequencing following standard protocols (see website for details: http://functionalbio.com/web/index.php).

Bidirectional sequences were obtained for six of the eight samples sent for processing. Sequences were imported into CodonCode Aligner version 3.7.1 (CodonCode Corporation Centerville, Massachusetts, USA). Forward and reverse sequences were

edited prior to building consensus sequences. Edited consensus sequences were exported to Geneious Pro version 4.8.5 (Biomatters Ltd. Newark, New Jersey, USA) for alignment on default settings. Final sequences were submitted to Genbank (Benson *et al.,* 2012).

All sequences generated were pooled and additional *rbcL* sequences for *B. laevigata* (DQ006074.1) and *Draba nemorosa* (NC009272.1) were imported from Genbank (Benson *et al.,* 2012). An alignment of all eight sequences was generated using the MUSCLE alignment program as a plug-in in Geneious Pro vers. 4.85 (Biomatters Ltd. Newark, New Jersey, USA). Genetic distance was visually inspected by generating a Tamura-Nei neighbor joining tree with a 90% support threshold using *Draba nemorosa* as an outgroup (Drummond *et al.,* 2011).

Assessing genetic variation

To determine possible variation in ploidy within and among populations young leaf tissue from eight individuals was collected from *ex situ* genetic stock plants representing two separate populations of *A. georgiana* (Table 6). Fresh leaf tissue was sent on ice to the University of Guelph, for analysis using flow cytometry. Tissue quality degradation was noted upon receipt (Kron, 2012). Upon arrival, leaves were kept cool and moist until testing was conducted (Kron, 2012). Genome size estimates followed the protocol of Kron 2012 (Appendix B). Nucleic DNA content was calculated as (peak mean of test plant) / (peak mean of standard) x (DNA content of standard) (Kron, 2012).

To assess haplotype variation using the whole chloroplast *trnL* (UAA) intron, total genomic DNA was isolated from 48 samples of *A. georgiana* using the methods previously outlined. Samples represented 6 of the 10 populations studied (Table 7). The

forward primer *trnL* C and reverse primer *trnL* D were used (Table 5) (Taberlet *et al.*, 2007). Reagents were combined in 20µl reaction mixture containing 0.2µl of 5U/µl of AmpliTaq Gold Taq polymerase (company info), 2.4µl of 10X AmpliTaq Gold buffer, 2µl of 25mM $MgCl_2$, 2µl of 2mM premixed dNTP's (Applied Biosystems, Branchburg, New Jersey, USA), 0.8µl of 10µM of each primers, 9.8µl nuclease free dH_2O, and 2µl of template DNA.

The following protocol was used to amplify the *trnL* (UAA) intron: initial denaturation at 95.0 °C for 5 minutes, 35 cycles of 95.0 °C for 1 minute, annealing at 38.0 °C for 45 seconds, extension at 72.0 °C for 1 minute, followed by a 10 minute final extension at 72.0 °C, and a final hold at 4.0 °C. Unpurified PCR product was shipped on ice to Functional Biosciences Inc. for sequencing (Madison, Wisconsin, USA). Sequences generated from the *trnL* (UAA) intron were edited in the same manner as *rbcL* sequences described earlier.

To compare *A. georgiana's* haplotype variation to that of other congeners, sequences for *A. blepharophylla* (FJ188288.1), *A. pycnocarpa* (FJ188198.1; FJ188213.1) and *A. patens* (FJ188264.1; FJ188152.1) were imported from GenBank (Benson *et al.*, 2012). A Tamura-Nei neighbor-joining tree was constructed using all *A. georgiana* sequences and those sequences downloaded from GenBank (Benson *et al.*, 2012) with *A. blepharophylla* as an outgroup. Default settings were used with the exception of support threshold, which was set to 80% (Drummond *et al.*, 2011).

To identify microsatellite loci that may be useful for screening *A. georgiana* populations, seven loci previously identified as being amplifiable and polymorphic in a number of other species of Brassicaceae (Skrede *et al.*, 2009) were screened for

amplification: AthCTRI, AthSO392, DnA214, DnB101, DnB123a, DnB220, and MR187 (Table 5). An initial screening for amplification was performed according to the protocols of Skrede *et al.* (2009). Five loci showed positive amplification during initial testing: AthCTRI, AthS0392, DnA214, DnB123, and DnB220. To test for polymorphy, primer sequences for loci showing positive amplification were sent to Ecogenics (GmbH, Zurich-Schlieren, Switzerland) along with genomic DNA from a subsample of 15 *A. georgiana* individuals. Of the five loci screened, all amplified but only two showed evidence of polymorphy suitable for assessing population structure in *A. georgiana* (Table 8). Based on these results, two loci, DnA214 and DnB220, were selected for further testing.

Total genomic DNA was isolated from a 101 samples of *A. georgiana* representing 10 populations (Table 9). DNA extraction from all samples were sent to Ecogenics (GmbH, Zurich-Schlieren, Switzerland) for microsatellite analysis using the loci DnA214 and DnB220. Multiplex amplification used the following protocol: 10µL reaction volume containing 5-10ng DNA, 5µl HotstarTaq Master Mix (Qiagen, Cat. No 203445) providing a final concentration of 0.5 units HotStarTaq DNA polymerase, 1X PCR buffer with 1.5mM $MgCl_2$ and 200µM of each dNTP. Additionally, 1.8µl nuclease free dH_2O and 0.3µM of both forward and reverse primers were used for each reaction. Unlabeled forward and reverse locus primers were mixed with labeled primers prior to PCR so that following PCR, product could be loaded for sequencing without additional dilution.

PCR thermotreatment for multiplexing was as follows: initial denaturation at 95.0 °C for 15 minutes, 35 cycles at 94.0 °C for 30 seconds, 48.0 °C for 90 seconds and 72.0

°C for 1 minute, followed by a 30 minute extension at 72.0 °C. Following amplification, 1.2µl of amplified PCR product was mixed with 10µl nuclease free dH_2O containing GENESCAN-500 (LIZ) size standard (Applied Biosystems, Branchburg, New Jersey, USA). Genotype was then determined using an ABI Prism 3730 Genetic Analyzer using GeneMarker® Software version 1.80 (SoftGenetics LLC®, State College, Pennsylvania, USA). Run conditions for genotyping with dye were as follows: injection time 10 seconds, injection voltage 1.6 kV, run time 2100 seconds, run voltage 15kV, capillary length 50cm with POP7 polymer.

Fragment analysis chromatogram (FSA) data files generated were scored using Gene Mapper version 4.0 on default settings (Applied Biosystems, Branchburg, New Jersey, USA) (Fig. 11). When examined for scoring, potential variation in locus DnA214 was revealed to be from artifact peaks. All 101 samples shared identical alleles. Because this locus was not informative, it was excluded from further analysis. Data for locus DnB220 was then uploaded into GenAlEx version 6.41 (Peakall and Smouse, 2006). Alleles were listed as codominant with the population flag set to zero and then exported to STRUCTURE version 2.3.3 for analysis using a Bayesian clustering method (Pritchard *et al.,* 2000). Length of Burnin period was set to 5,000 with 50,000 Markov Chain Monte Carlo (MCMC) repetitions. Parameters were set to default using a no admixture model and prior population information. These choices were selected based on known fragmentation and location of populations which make it unlikely that either gene flow or migration could occur between *A. georgiana* populations. STRUCTURE analysis genetic cluster (K) values were set from 1 to 10 with 10 iterations.

Results generated by STRUCUTRE were imported into STRUCTURE HARVESTER (Dent & vonHoldt, 2012) so that estimates of K using the Evanno *et al.* (2005) method could be generated. This method was selected due to its assumption that known populations may not be at Hardy-Weinberg equilibrium due to recent fragmentation or reduction in size – a reasonable assumption for the remaining population of *A. georgiana* included in this analysis.

Once an estimate of K was generated, data was loaded back into GenAlEx to assess the fixation index (F) and the unbiased Nei genetic distance between populations. GenAlEx was designed to generate information for microsatellite data for diploid organisms. Although *A. georgiana* is not diploid, the single marker available for genetic analysis presents only two peaks per individual. Preliminary analysis revealed no variation in ploidy among individuals representing two populations. Therefore, estimates of population structure using GenAlEx were deemed to be suitable for this study.

RESULTS

Population census

Comparisons of current population size to values generated during the 2005 census cannot be made for many of the Alabama populations due to a lack of data (Moffett, 2007). For populations within Georgia or on Fort Benning, population numbers have either declined or remained stable since *A. georgiana* was originally listed as a species of concern in 1993 (Table 10). Fort Gaines, Whitmore's Bluff, and Black's Bluff have all decreased in population size. Decreases in population size at both Whitmore's

Bluff and Fort Gaines were significantly greater than expected due to chance ($X^2 = 28.88$, d.f. = 1, $p < 0.0001$; $X^2 = 6.34$, d.f. = 1, $p < 0.01$).

The Georgia populations of *A. georgiana* on both Fort Benning and Oostanaula Bluffs appear stable (Table 10). The Fort Benning population revealed almost identical numbers of *A. georgiana* at the time of the 2010 census as compared to the 2005 census ($X^2 = 0.05$, d.f. = 1, $p > 0.05$). The increase in population size at Oostanaula Bluffs was not significantly different between censuses ($X^2 = 3.13$, d.f. = 1, $p > 0.05$). Census counts for the Goat Rock and Ft. Benning, Alabama populations significantly increased ($X^2 = 1746.79$, d.f. = 1, $p < 0.00001$; $X^2 = 1173.43$, d.f. = 1, $p < 0.00001$). These increases may represent recruitment, shifts in age structure of populations, or more thorough population searches.

The naturally occurring population at Black's Bluff decreased in size. All naturally occurring plants are extirpated from this site. The increase in plants reported at Black's Bluff is due to surviving plants from an augmentation effort executed in 2010 (Georgia Native Plant Society, 2010). No Chi-Squared goodness-of-fit test was performed for this population due to this confounding variable.

Confirmation of genetic identity

The *rbcL* sequences generated for *A. georgiana* and *B. laevigata* were discrete from one another but highly conserved within each species (Fig. 12). Consensus sequences varied by six base pairs between species. Consensus support for grouping species in separate clades was 100%. This sequence data supports the hypothesis that *B. laevigata* and *A. georgiana* are distinct species from one another.

Assessing genetic variation

Sample tissue from seven individuals analyzed by flow cytometry provided a mean genomic DNA content of 2.69 (+-0.0193) pg/2c. (Kron, 2012). True members of the genus *Arabis* should have a base chromosome number of 8 (Al-Shehbaz, 2001; Johnston *et al.,* 2005; Warwick and Al-Shehbaz., 2006). When the 2c value generated for *A. georgiana* is compared to 2c content of other members of the Brassicaceae with a base chromosome number of 8, a preliminary designation of *A. georgiana* as an octoploid species can be made (Koch *et al.,* 2000; Dart *et al*., 2004; Grundt *et al*., 2005; Johnston *et al.,* 2005; Warwick and Al-Shehbaz, 2006; Koch *et al.,* 2010; Kron, 2012) (Table 11).

There was no variation present within 48 *trnL* (UAA) intron sequences generated for *A. georgiana.* This gene region provides no information about relationships between remnant population clusters for this species. Sequences of *A. pycnocarpa* downloaded from GenBank were a 100% match to *A. georgiana* (Table 12). Although for approximately 25%-35% of plant species, this region provides no discrimination between species (Taberlet *et al.,* 2007; Gonzalez-Varo *et al.,* 2009), *trnL* sequence data supports the hypothesis posited by Koch *et al.* (2010) that *A. pycnocarpa* could be an ancestral species to *A. georgiana.*

Of the two microsatellite regions screened, only locus DnB220 showed true variation. Potential variation observed during screening at this locus was revealed to be due to artifact peaks. All 101 samples were identical at locus DnA214 (Fig. 11). Locus DnB220 had two alleles per individual with four alleles in total. Estimates generated using STRUCTURE version 2.2, followed by STRUCTURE HARVESTER, suggest that

A. georgiana is composed of either two or three genetic clusters (K) or populations. The mean Delta K value for two populations was slightly higher than for three populations at 0.65 versus 0.62 respectively. Calculated posterior probability values plateau at this point and then drop sharply indicating that either two or three genetic clusters is most likely for this species (Pritchard & Wen, 2004). Based on physical distribution of sample sites, and observed homogeneity aligned with geography, K of three was used for further analysis. Each genetic cluster was fixed for the loci used for analysis but, groups have different alleles from one another. The North Georgia populations, which hold the 190 allele, consist of Whitmore's Bluff, Black's Bluff and Oostanaula Bluffs. South Georgia populations consisting of Goat Rock, both Fort Benning populations and Fort Gaines, comprise the second group and hold the 184 allele. The Alabama group is composed of populations which hold the 192 allele which include Durant's Bend, Prairie Bluff and Pratt's Ferry. All individuals from each population shared at least one copy of allele 186 and some individuals within the South Georgia genetic cluster were homozygous for this allele (Table 13). A single Alabama population occurring on Fort Benning was grouped in with the South Georgia genetic cluster. This was done based on the physical location of the populations, K values generated by STRUCTURE HARVESTER, and an assessment of genetic distance between populations. When unbiased Nei genetic distance was calculated with K = 4, using the Fort Benning, Alabama population as an additional genetic cluster, results indicated that this population was more closely aligned with the South Georgia than the Alabama genetic cluster (Table 14a).

The distance between the three major genetic clusters revealed by unbiased Nei genetic distance calculations, was 0.47 between North Georgia and South Georgia

genetic clusters, 0.48 between South Georgia and Alabama and 0.68 between North Georgia and Alabama (Table 14b) (Fig. 13).

The fixation index (F) for this species was calculated using GenAlEx. F values generated in GenAlEx can range from -1 to 1 (Peakall and Smouse, 2010). If a population is close to Hardy-Weinberg equilibrium the value should be close to 0o. A value close to 1 indicates inbreeding while a value close to -1 indicates a possible selection advantage for heterozygotes (Peakall and Smouse, 2010). The F values calculated for the North Georgia, South Georgia and Alabama populations were -1.00, -0.58 and -1.00 respectively.

DISCUSSION

This study represents one of the first attempts to quantify population distribution, size and the genetic identity of *A. georgiana.* Census data revealed population growth, stability and decline at various populations although no species-wide trend was apparent. Analysis of species identity through the use of the *rbcL* barcoding region confirms that *A. georgiana* is a distinct species. Analysis of genomic DNA content using flow cytometry showed no variation in ploidy across the species range and suggests that *A. georgiana* is most likely octoploid. Although no sequence variation was found among *trnL* (UAA) intron cpDNA haplotypes, a variable microsatellite locus revealed significant genetic structuring across populations. These results are discussed below and provide a valuable first assessment of the genetic identity and structuring of this species.

Population census

Census information was collected to help establish baseline information about known populations of *A. georgiana.* A consistent pattern of growth or decline between 2005 and 2010 was not apparent. The Goat Rock, GA and Fort Benning, AL populations increased during the 2010 - 2011 census period. While dramatic fluctuations in population size are not unknown for endemic taxa such as *A.* georgiana (Falk & Holsinger, 1991), increases to census counts for the Fort Benning, Alabama complex probably represent a more thorough search covering a larger area. This population is on federal lands where it has been afforded protection since time of listing. The increase in census numbers at the Goat Rock population is likely due in part to the year censused rather than permanent recruitment. Most of the plants observed at this population were juvenile rosettes which appeared to be under six months old. This is in contrast to the age structure of the population observed during the previous two years when the majority of plants observed were either large vegetative rosettes or reproductive individuals. Field observations indicate that juvenile mortality is high (Garcia *et al.,* 2011) for this species and that a classic type III survivorship curve with high reproductive output and few individuals surviving to a reproductive stage, may best describe the survival strategy for this species.

Although the Fort Gaines, GA population appears to have significantly decreased between 2005 and 2010 a more comprehensive survey of this site is needed to verify these results. The area surveyed during this study may consist of a different patch of plants than those counted during 2005. Sites censuses conducted during this study were aligned with ecological occurrence data obtained from The Georgia Plant Conservation Alliance (2010). However, subsequent information obtained from The Georgia

Department of Natural Resources indicates that more than one patch of plants may be present at this site (M. Moffett, *personal communication* 2012). The full extent and distribution of the Fort Gaines population requires additional investigation.

For populations such as Black's Bluff, which is now naturally extirpated, and Whitmore's Bluff, declines in population size could be cause for concern. While at the time of the 2005 census Black's Bluff had only three individuals, Whitmore's Bluff still supported 50. Based on the sharp decline in plants found at this population, it may be reasonable to assume that the observed decline in population size within this population has dipped below required levels for long term viability. When current population sizes for these populations are considered in light of minimum viable population size estimates, the likelihood of long-term survival for either population appears grim (Traill *et al.,* 2007; Jamieson and Allendorf, 2012).

Confirmation of genetic identity

The genus *Arabis* is currently in flux and exact taxonomic relationships are still being evaluated (Weakley, 2012, Koch *et al.,* 2010, Al-Shehbaz, 2003). The *rbcL* sequences generated for both *B. laevigata* and *A. georgiana* support the current taxonomic standing of these as distinct species. These two species do bear a morphological resemblance and co-occur but, there is no evidence of any deeper relationship. While this is not surprising given that true members of *Arabis* and *Boechera* differ in base chromosome number (Al-Shehbaz, 2003), the generation of a novel barcode for *A. georgiana* using one of the loci recommended by the Consortium for the Barcode of Life (CBOL Plant Working Group: Hollingsworth *et al.,* 2009) may prove useful for

future confirmation of newly discovered populations without the need for reproductive structures (Burgess *et al.,* 2011).

Assessment of genetic variation

Genome size estimates generated through flow cytometry suggest that *A. georgiana* is octoploid (8x) and DNA content did not vary among samples collected throughout the range. However, genome size estimates generated using flow cytometry are from a small number of samples and should not be considered as the final estimate of genome size for this species nor a final verification of the lack of variation among sites. Furthermore, visual verification of chromosome number is needed. The preliminary estimate of *A. georgiana* as octoploid is interesting when considered in relation to work by Koch *et al.* (2010) where it was hypothesized that *A. georgiana* evolved as a byproduct of ancient hybridization between *A. patens* and *A. pycnocarpa.* ITS data generated in that study supported that *A. georgiana* shared a close phylogenetic relationship to *A. patens.* Both hypothesized parental species of *A. georgiana* are generally tetraploid (4x) although some individuals of *A. pycnocarpa* are known to be octoploid (8x) (Koch *et al*., 2010; Warwick and Al-Shehbaz, 2006). The preliminary identification of *A. georgiana* as octoploid is significant when considered in relationship to possible parental taxa and may explain the larger size of certain diagnostic structures, such as fruit, belonging to *A. georgiana* (Patrick *et al.,* 1995). Although this finding is significant both in understanding the evolution of this species and chromosome evolution in the Brassicaceae, estimates of genome size did not reveal any genetic structuring among remaining populations of *A. georgiana.*

Within the *A. georgiana* samples analyzed there was no sequence variation in the *trnL* (UAA) intron among *A. georgiana* samples collected across the species range. Furthermore, sequences generated for the *trnL* intron showed that *A. pycnocarpa* was an exact sequence match to *A. georgiana* for this region. While shared *trnL* sequences between separate species of Brassicaceae are known (Sharbel & Mitchell, 2001), the close relationship in the *trnL* sequence discovered between *A. georgiana* and *A. pycnocarpa* provides valuable insight into potential ancestral relationships of *A. georgiana* and supports the hypothesis that *A. pycnocarpa* is an ancestral species to *A. georgiana.* Given the lack of interspecific variation among species, the fact that the *trnL* intron region did not reveal any population structuring for *A. georgiana* is not surprising. This indicates that although there are high levels of fragmentation between populations of this species, fragmentation may have occurred too recently for any sequence substitution to occur among populations for this particular chloroplast gene region.

Trans-genus transfer rates of 6-18% for microsatellites listed by Skrede *et al.* (2009) were upheld for *A. georgiana*. Of seven primer sets tested, one was variable. Primers for locus DnB123 may also merit further testing. Analysis of variation at locus DnB220 did allow some general information about population structure for this species to be generated. Based on genetic data from 101 individuals, representing 10 populations, it is evident that there is genetic structuring which seems to be based more on geographic location than on population size. However, this information must be considered cautiously as it is based on data from one variable microsatellite loci and further confounded by the finding that *A. georgiana* is most likely an octoploid species.

The Nei genetic distance calculations showed that the North Georgia and South Georgia genetic clusters had the closest relationship (0.47) indicating the lowest amount of time since these populations were isolated from one another. The South Georgia and Alabama genetic clusters were intermediate at 0.48 while the North Georgia and Alabama populations were the most distant from one another (0.68). These findings could be interpreted in multiple ways. First we could assume that the similarity within the genetic clusters and variation between them is an indication that these were once three large and separate populations which have only been separated within recent history. Results could also be interpreted to mean that habitat fragmentation has led to genetic drift, causing individuals within a population to become genetically homogenous. This interpretation does not adequately explain why there is a greater than expected proportion of heterozygotes present in the samples assessed. F values of -1.00, -0.58 and -1.00 for North Georgia, South Georgia and Alabama populations respectively, are well below what would be expected unless a heterozygote advantage is present at the loci evaluated. Only 11 of the 101 *A. georgiana* samples evaluated were homozygous at the particular gene region examined. However, fixation for the homozygote condition within all Fort Benning, AL samples may indicate that some genetic drift could explain the results observed.

As discussed earlier, *A. georgiana* is found within different geologic regions. It is possible that the genetic patterns discovered could confer a fitness advantage within particular regions due to local climactic patterns. However, some of the individuals within the Central Georgia genetic cluster are homozygous for allele 186 which was common to all 101 individuals. This means that even though the genetic variation present

between different populations may confer a fitness advantage, small population size leading to genetic drift is still an issue for this species. Genetic drift is also a threat for this species based on the extreme population subdivision suggested both by the physical locations and microsatetllite data. This phenomenon has been observed in many other species facing similar obstacles to long term survival (Lauterbach *et al.,* 2011; Wagner *et al.,* 2012; Li *et al.,* 2012).

Recommendations for management

According to the U.S. Endangered Species Act as amended (2002), genetically distinct populations merit protection under the law. Although *A. georgiana* is currently a federal candidate species for protection under this law (Norquist, 2009), it most likely merits protection as a threatened rather than endangered species due to the number of remaining populations and individuals. However, the North Georgia genetic cluster is in decline with regard to overall census numbers and possibly fitness which may be leading this genetic cluster into an "extinction vortex" as outlined by Newman & Pilson, 1997 and Keâry *et al.,* 2000. Given that this genetic cluster comprises the three northern most populations known for the species, it may be especially significant in the face of global climate change. Considering limited conservation funding and resources, efforts targeted at conserving the three North Georgia populations rather than more robust or abundant populations within other genetic clusters seems logical.

Due to the extremely small size of each of the North Georgia populations, mixing of population genotypes to counteract potential inbreeding depression has been suggested as a management strategy (Georgia Plant Conservation Alliance, 2012). Although these

populations do share a common genotype for locus DnB220, admixture of populations should still be considered cautiously. While mixing of North Georgia genotypes may be a valid conservation approach and provide the species with some relief from inbreeding depression, the assessment of population genetic structure revealed in this study is preliminary. A thorough population genetics study would evaluate at least 8 - 10 microsatellite loci in order to adequately assess population genetic structure (F_{st}) (Willams, 2001; Kalinowski, 2002). Furthermore, evaluation of additional samples from each population, such that at least 25 - 30 individuals per population or all individuals present, is needed in order to generate a satisfactory estimate of population genetic structure (Hale *et al.*, 2012).

The Black's Buff population in North Georgia is located on conservation land. As previously stated the natural population of *A. georgiana* at this site has been naturally extirpated. *Arabis georgiana* remaining at this population are progeny of one individual plant and have been generated through conservation breeding and restoration efforts. If retention of the species at the Black's Bluff site in North Georgia is a primary conservation goal then establishing a mixed North Georgia population at this site may merit consideration. If this approach for conservation of these perilously small populations is chosen it would allow the conservation community to study the effects of gene flow between populations.

More study is needed to determine true levels of genetic structure for this species. If other variable gene regions reveal similar results to those found in this study, then some assisted migration between select populations might help restore an intermediate

amount of genetic variation between populations which may ultimately lead to the greatest evolutionary potential for *A. georgiana.*

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FIGURES

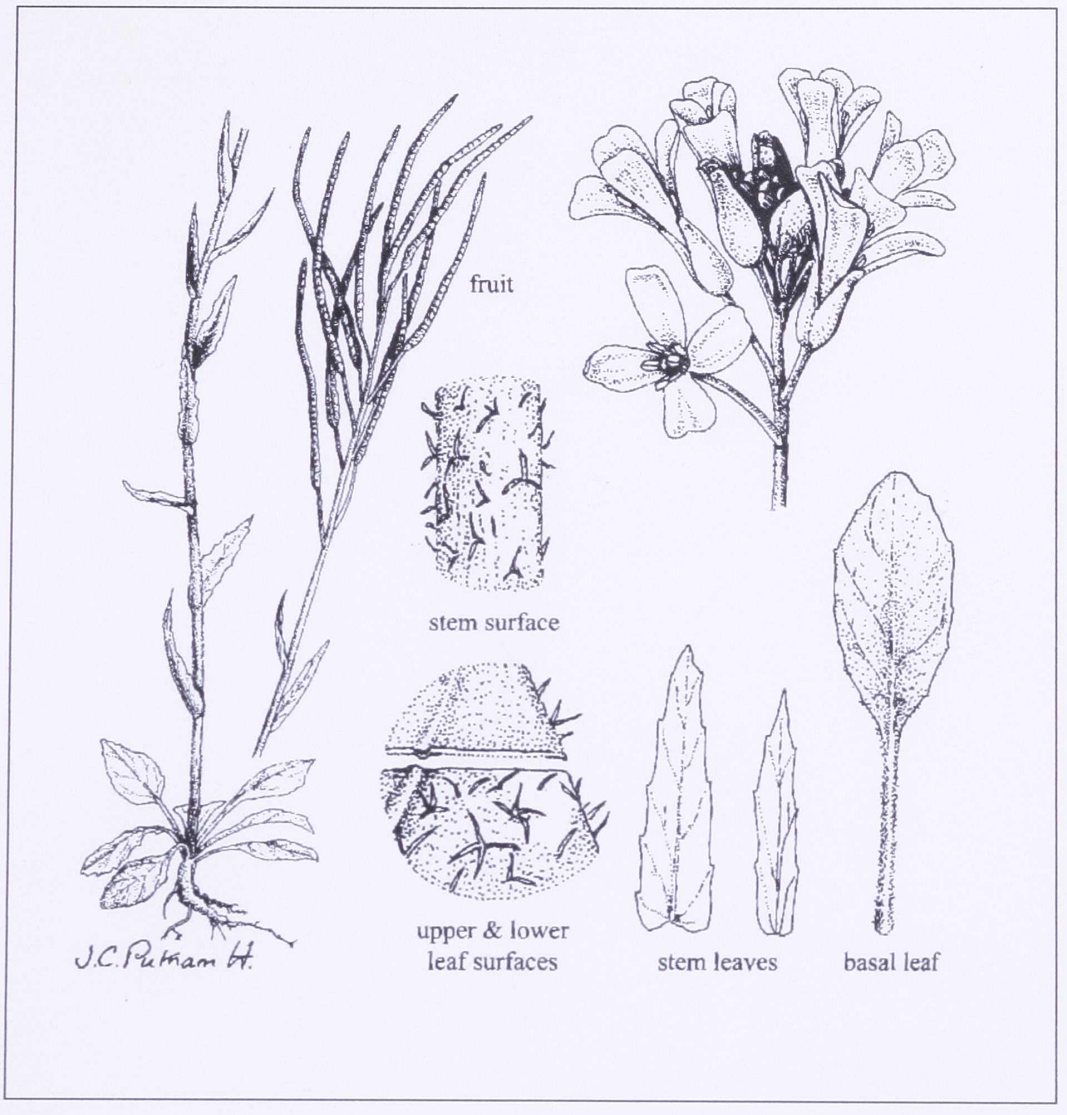


Figure 1: Line drawing of *Arabis georgiana* Harper (Georgia rockcress) by J.C. Putnam from Chafin, 2007.

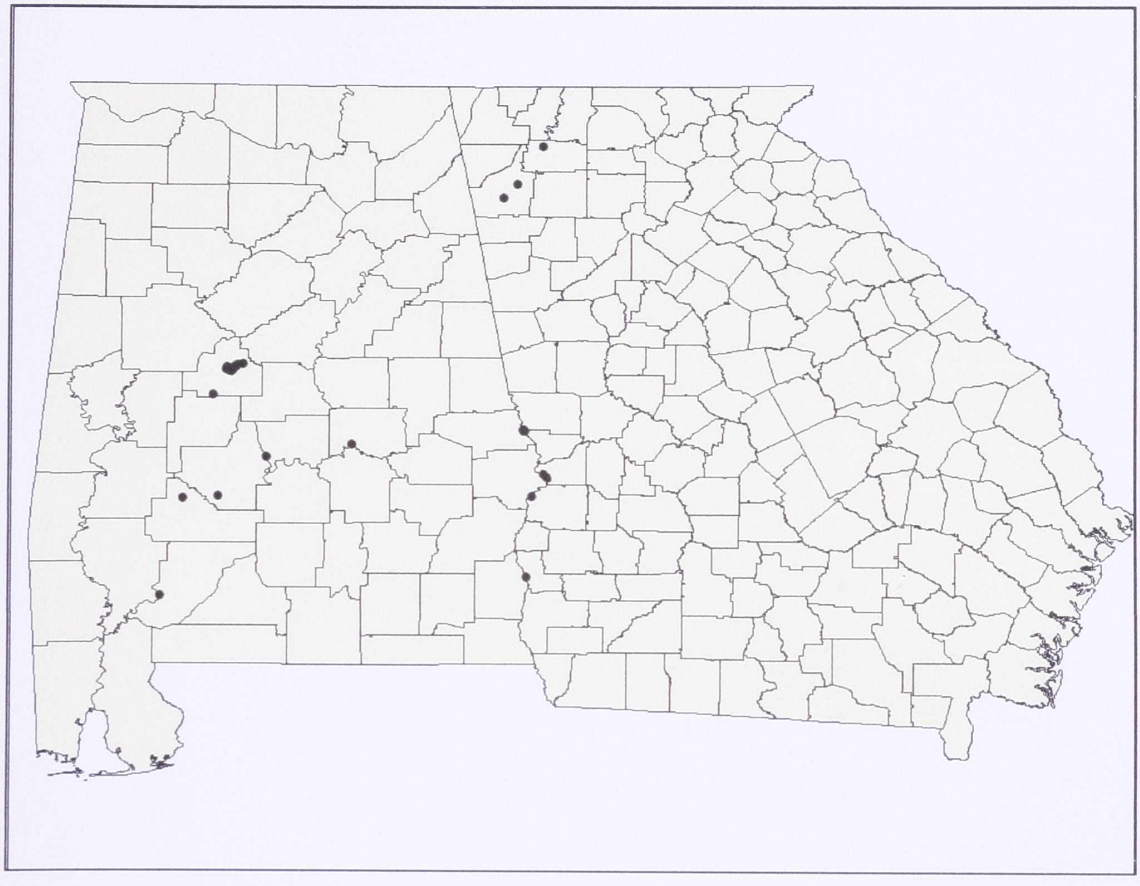


Figure 2: Locations of 17 remaining populations of *Arabis georgiana,* which is endemic only to Alabama and Georgia.

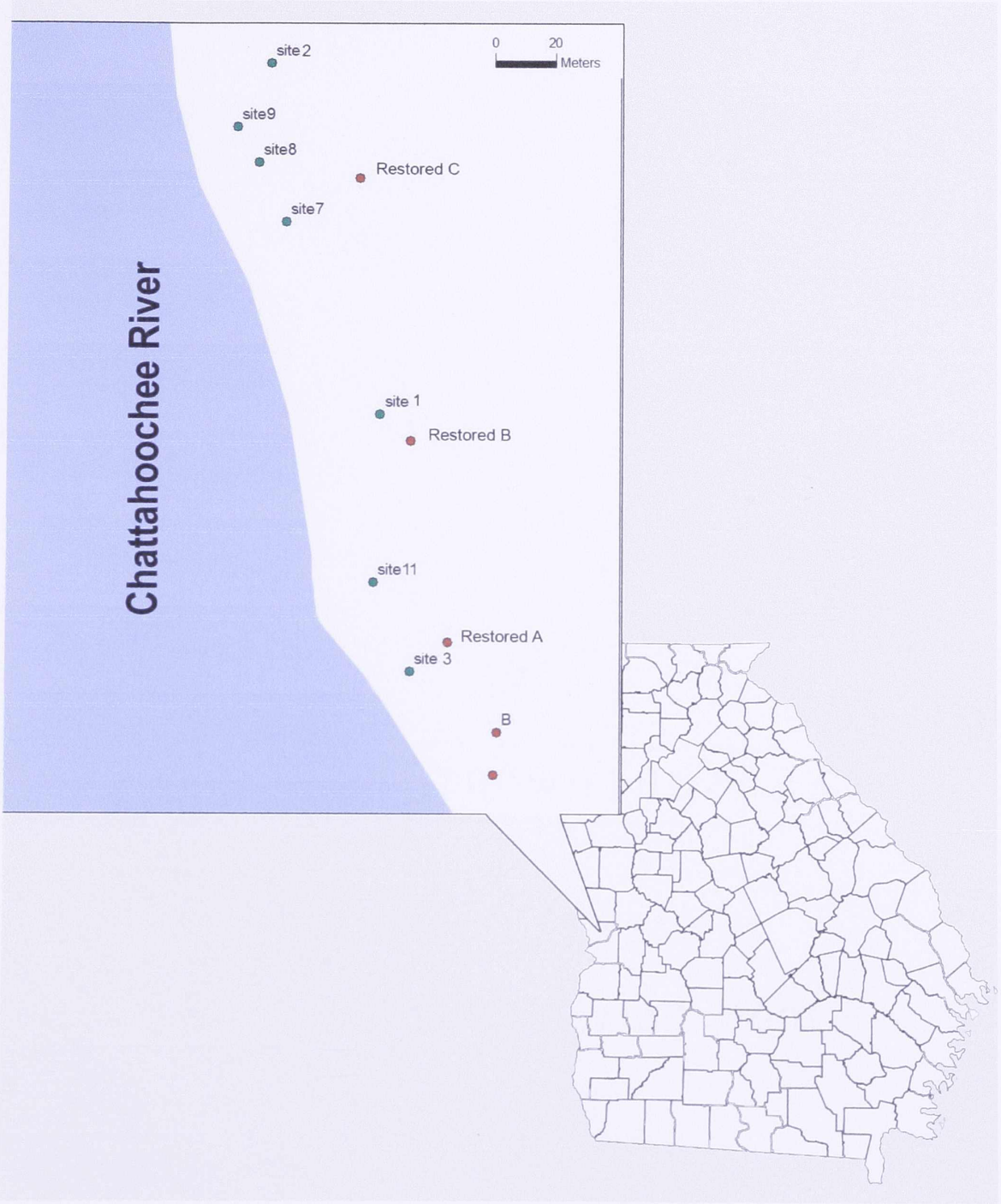


Figure 3: Spatial distribution of both long term native monitoring plots and restored plots at the Harris county *Arabis georgiana* study site.

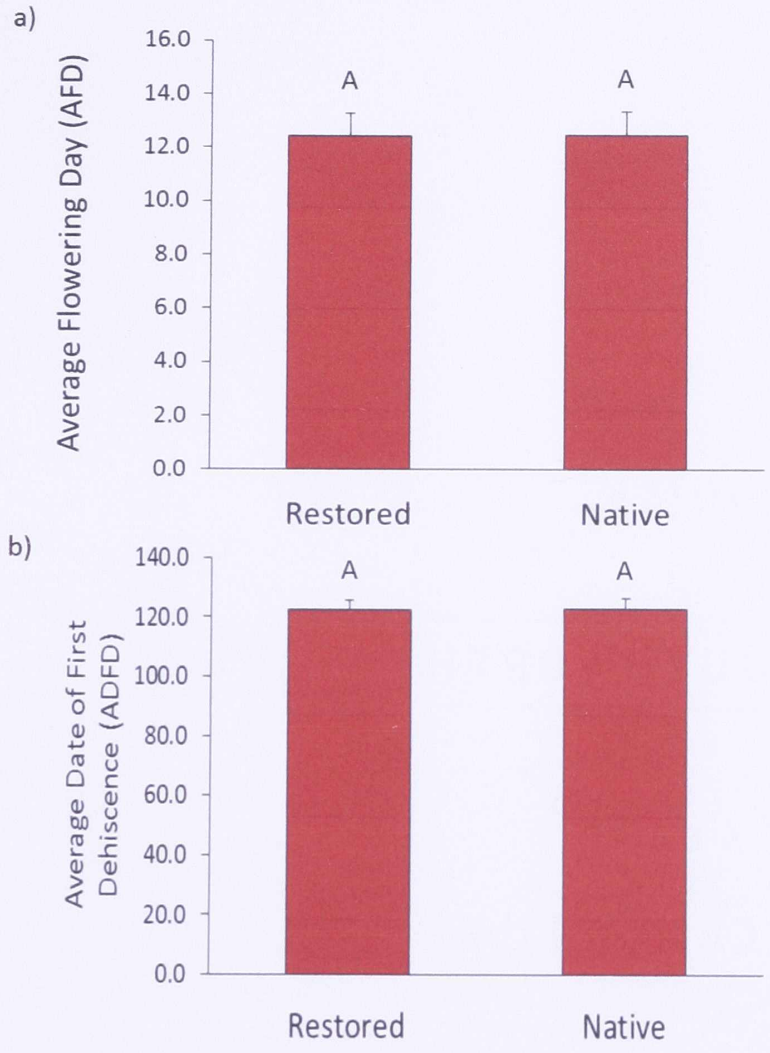


Figure 4: Traits measured to evaluate differences in phenological progression between restored and native plot types: a) average flowering day ($F_{1,76} = 0.09$, $p > 0.05$); b) average date of first dehiscence ($F_{1,76} = 0.004$, $p > 0.05$).

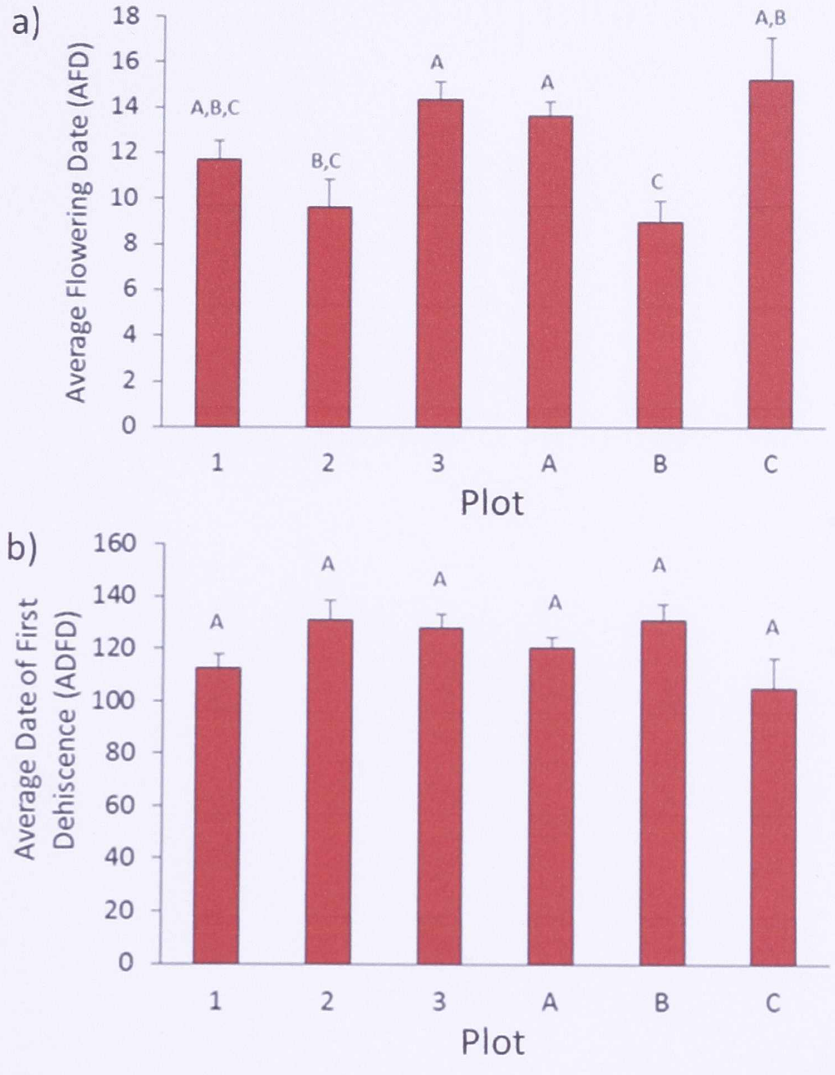


Figure 5: Analysis of traits measured by plot to evaluate differences in phenological progression between restored and native plot types: a) average flower date ($F_{5,72} = 7.57$, $p < 0.0005$); b) average date of first dehiscence ($F_{5,72} = 1.72$, $p > 0.05$).

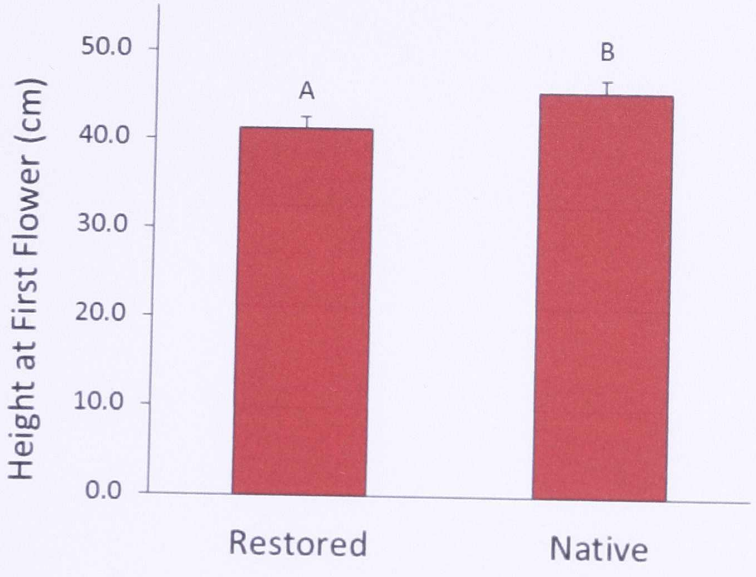


Figure 6: Differences in height at first flower between restored and native plot types. Restored plants were significantly shorter than native plants ($F_{1,76} = 5.05$, $p < 0.05$).

Figure 7: Differences in height at first flower between plots. There were no significant differences between plots ($F_{5,72} = 1.72$, $p > 0.05$).

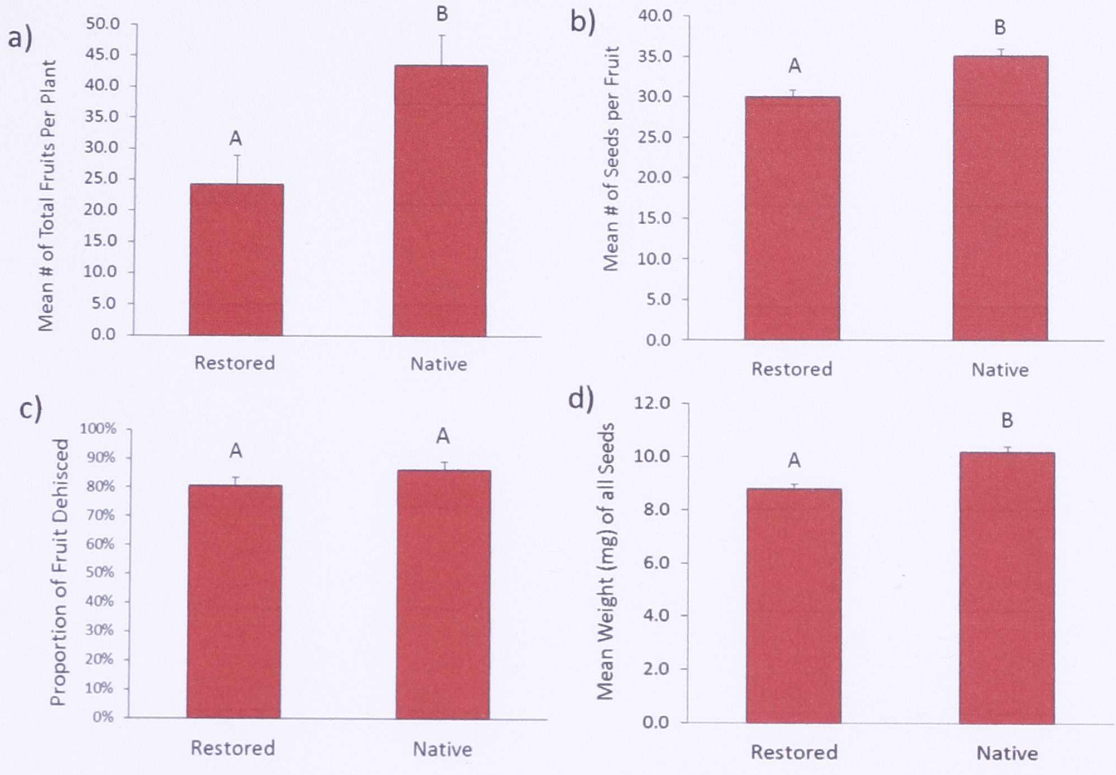


Figure 8: Variation in traits associated with reproductive success between native and restored plot types: a) mean number of fruit produced ($F_{1,76} = 10.93$, $p < 0.001$); b) mean number of seeds per fruit ($F_{1,76} = 17.64$, $p < 0.0001$); c) proportion of fruit dehisced ($F_{1,76} = 2.69$, $p > 0.05$); d) mean weight of total seeds from two fruits per plant ($F_{1,75} = 3.89$, $p < 0.01$).

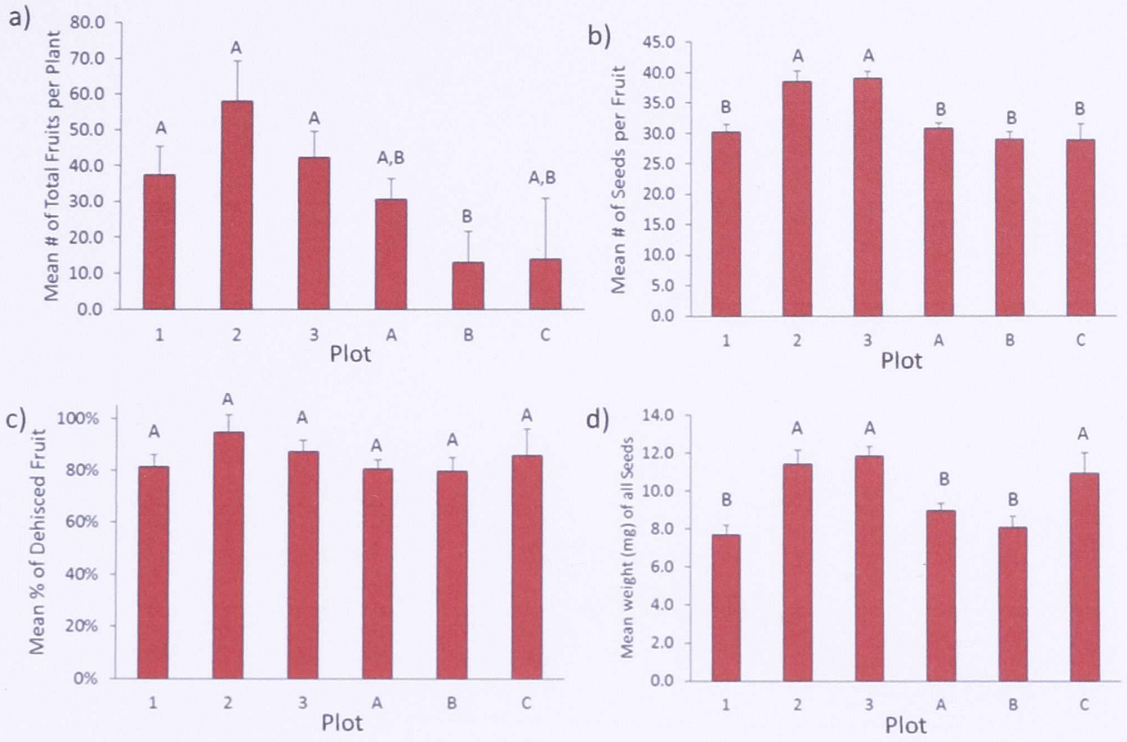


Figure 9: Variation in traits measured to assess differences in reproductive success between plots: a) mean number of total fruits per plant ($F_{5,72}$= 4.66, $p < 0.005$); b) mean number of seeds per fruit ($F_{5,72}$ = 11.94, $p <.0001$); c) mean proportion of fruit dehisced ($F_{5,72}$ = 1.58, $p > 0.05$); d) mean weight of total seeds from two fruits per plant ($F_{5,71}$ = 11.2, $p < 0.001$).

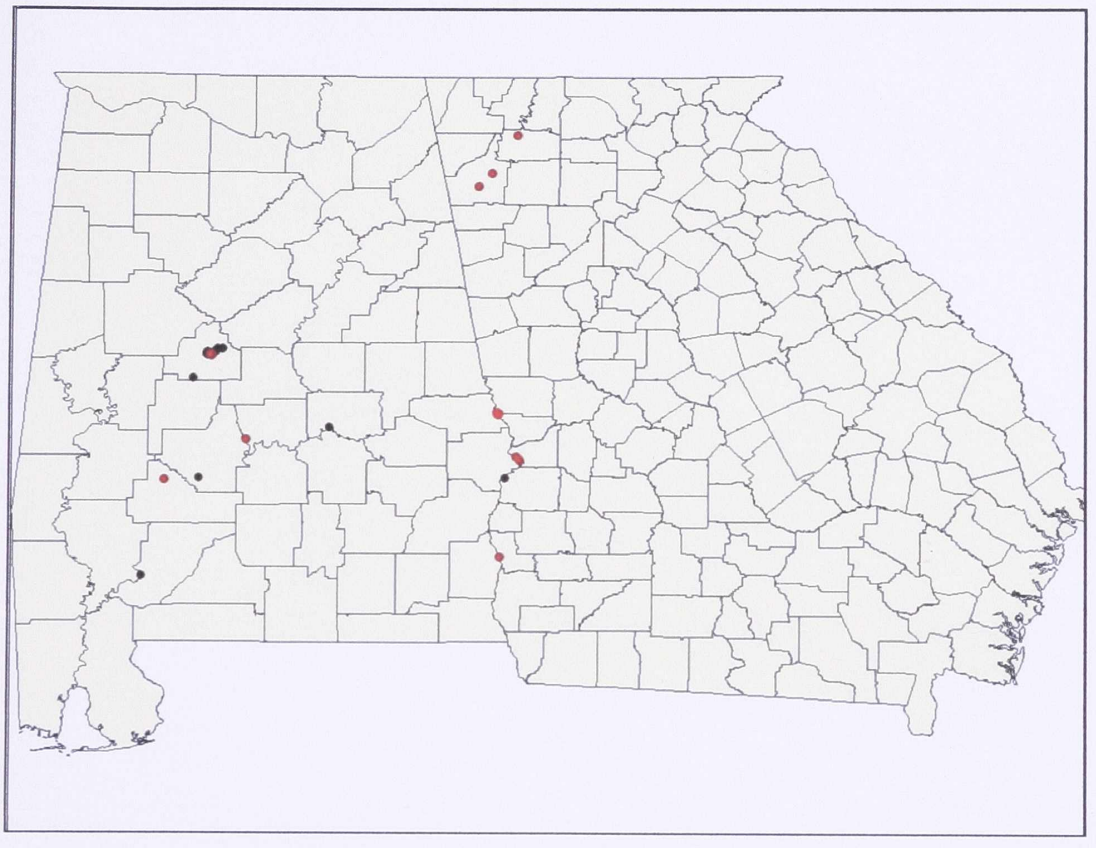


Figure 10: Locations of 17 remaining populations of *Arabis georgiana* endemic only to Alabama and Georgia. Populations indicated by a red dot were sampled for genetic analysis of population structure.

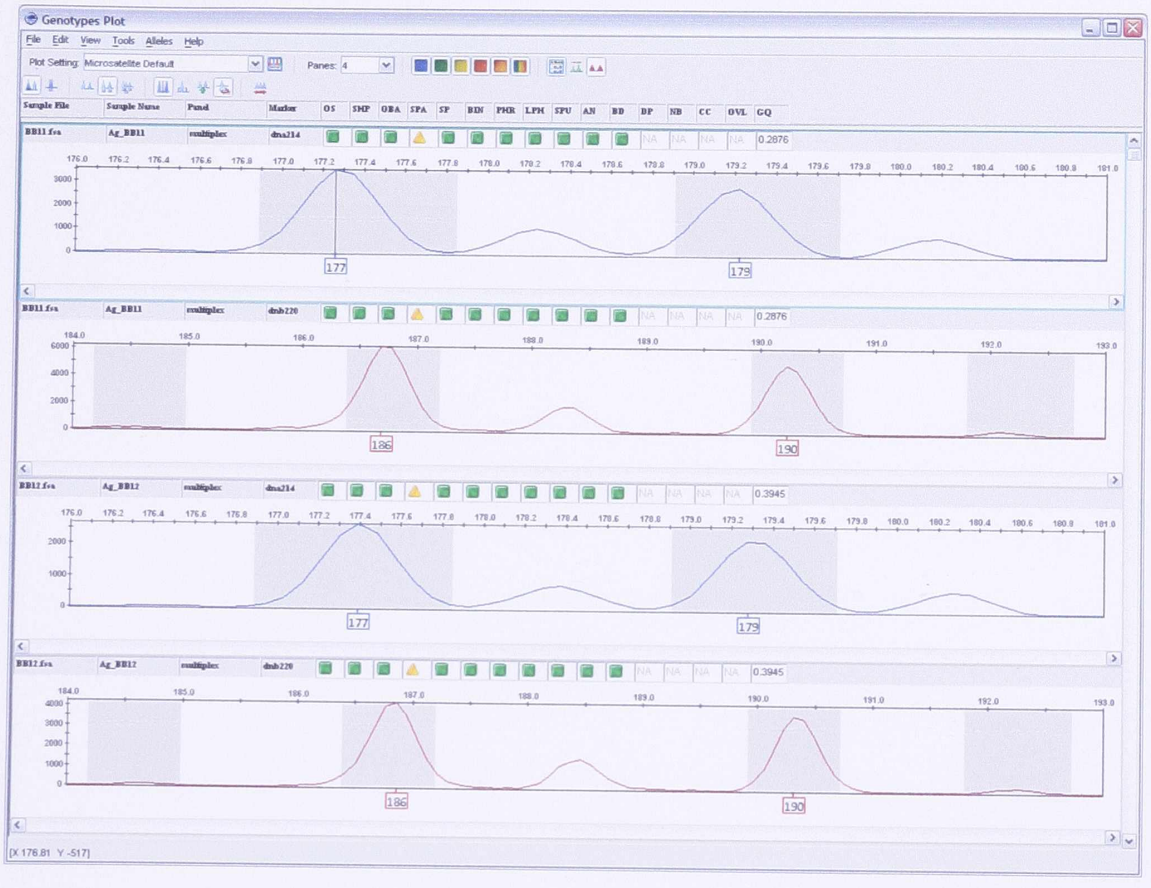


Figure 11: Examples of FSA file peaks for two *A. georgiana* samples (Gene Mapper 4.0). Peaks shown in blue are from locus DnA214 which was homozygous for all individuals screened. Peaks shown in red are from locus DnB220 which had one variable allele and revealed four distinct genotypes within the 101 samples screened.

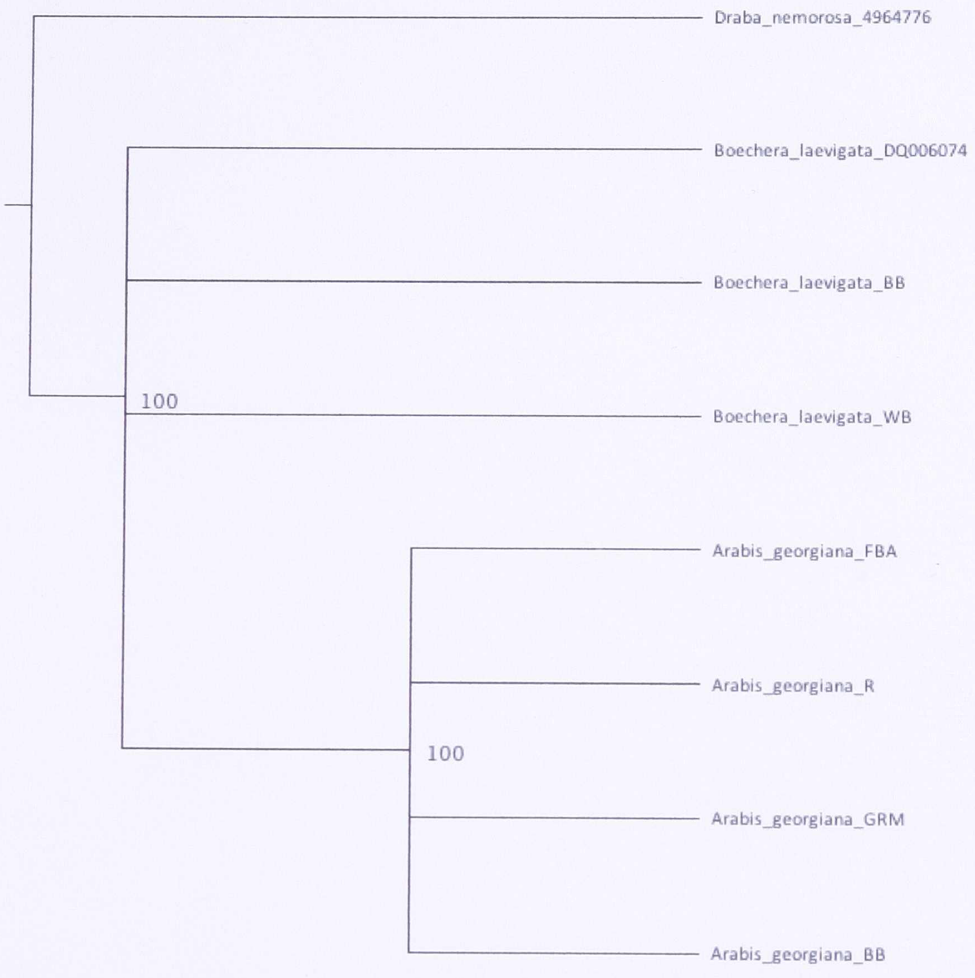


Figure 12: Tamura-Nei neighbor joining tree for *Arabis georgiana* and *Boechera laevigata rbcL* sequence haplotypes. A 90% threshold grouped the two species as separate clades with 100% consensus support (Drummond *et al.*, 2011).

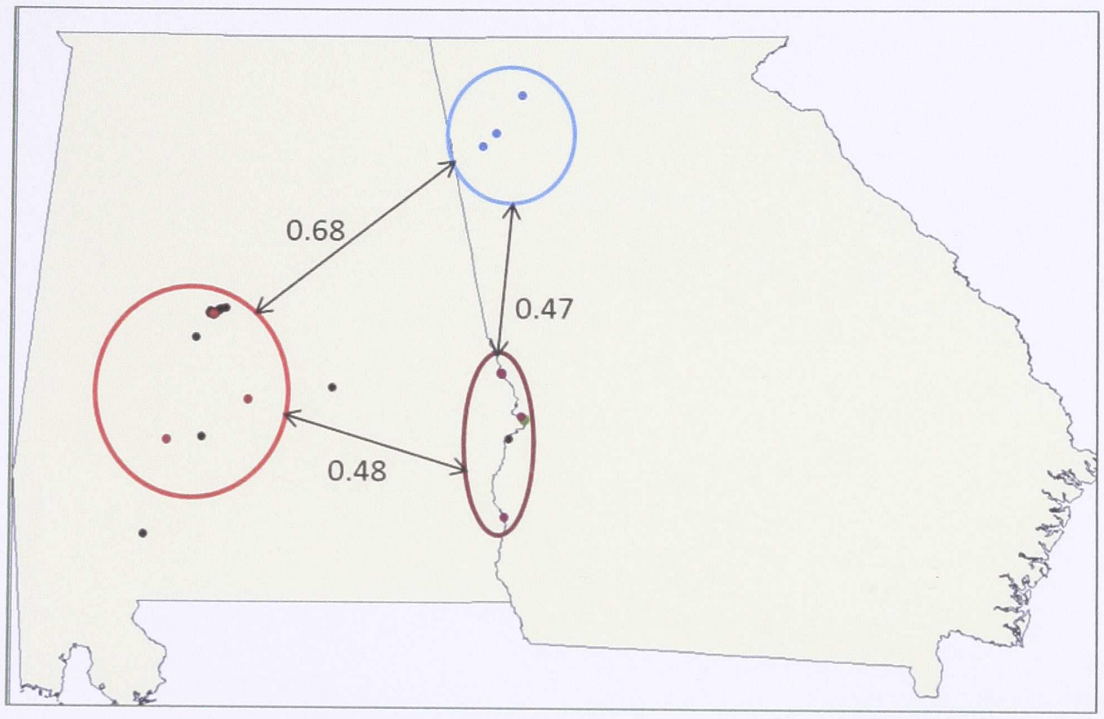


Figure 13: Microsatellite data from locus DnB220 revealed three genetic clusters for remaining populations of *Arabis georgina*: North Georgia (blue), South Georgia (brown) and Alabama (red). Unbiased Nei genetic distances between populations are indicated.

TABLES

Table 1: Results from a 2008-2010 census of *Arabis georgiana* plots at the Harris County, Georgia study site.

Plot Type	Plot	# Outplanted (Nov. 2008)	# Survived (Dec. 2008)	# Survived (Mar. 2009)	# Survived (Mar. 2010)
Restored	A	23	21	21	41
Restored	B	21	21	20	17
Restored	C	21	19	14	5
Native	1	-	-	8	16
Native	2	-	-	3	8
Native	3	-	-	17	26

Table 2: Soil analysis of restored vs native plots located in Harris County, Ga.

Trait measured	Restored plots (mean)	Native plots (mean)	ANOVA (F value)	Standard Error
pH	5.93	6.02	0.06	0.25
Calcium (g/m^2)	272.87	256.03	0.41	39.26
Potassium (g/m^2)	51.68	52.55	0.4	11.68
Magnesium (g/m^2)	31.48	25.87	0.86	8.72
Manganese (g/m^2)	6.1	5.01	0.99	1.28
Nitrate (NO_3) (g/m^2)	1.88	2.75	0.08	0.91
Phosphorus (g/m^2)	3.57	2.66	0.68	1.22
Zinc (g/m^2)	1.2	1.1	0.43	0.25

Table 3: Summary of reductions in performance for restored plots (expressed as a percentage) when compared to native plots for traits associated with phenological progression and reproductive success. Significant reductions in trait performance for restored plots are indicated with an asterisk.

Days to first flower (AFD)	Days to first fruit dehiscence (ADFD)	Size	Number of fruit	Number of dehisced fruit	Number of seeds	Weight of all seeds in two fruits
0.0%	0.2%	*9.3%	**44.0%	6.5%	***14.4%	*13.0%

Table 4: Provenance of samples collected for genetic confirmation of *Arabis georgiana.* Sequence polymorphisms of the rbcL cpDNA barcode were evaluated using samples collected from populations where both *Arabis georgiana* and *Boechera laevigata* are known to co-occur as well as from populations where only *A. georgiana* is present. Sample with * is from Genbank.

Species	**Population Name**	**Both Species Present?**
Arabis georgiana	Balck's Bluff, GA.	Yes
Arabis georgiana	Fort Benning, AL.	No
Arabis georgiana	Goat Rock, GA.	No
Arabis georgiana	Resaca, GA.	Yes
Boechera laevigata	Black's Bluff, GA.	Yes
Boechera laevigata	Whitmore's Bluff, GA.	Yes
**Boechera laevigata* - DQ006074	Unknown	Unknown

Table 5: Primer sequences used to assess genetic variation in *Arabis georgiana*. Sequence polymorphism in the cpDNA genome was analyzed using forward and reverse primers for the *rbcL* gene region and the *trnL* (UAA) intron. Nuclear variation was assessed using five sets (forward and reverse) of microsatellite primers.

Primer Target	Primer Name	Sequence (5'-3')
rbcL gene region	*rbcL*a - Forward	ATGTCACCACAAACAGAGACTAAAGC
rbcL gene region	*rbcL*ajf634 - Reverse	AAACGGTCTCTCCAACGCAT
trnL(UAA) intron	*trn*L C - Forward	CGAAATCGGTAGACGCTACG
trnL(UAA) intron	*trn*L D - Reverse	GGGGATAGAGGGACTTGAAC
Microsatellite	DnB123a - Forward	CAGTGCAAAATGCGTGAAT
Microsatellite	DnB123a - Reverse	GCGTGGAGATAGAGAAAGAGC
Microsatellite	DnA214 - Forward	TTCGTCTTCTTGAGCACTGG
Microsatellite	DnA214 - Reverse	CGGAATTCAACCCCAATAGC
Microsatellite	DnB220 - Forward	GCAAAGCAGAGCGTAGAATGG
Microsatellite	DnB220 - Reverse	ACTCGGACGTCTCAATCAGC
Microsatellite	AthCTRI - Forward	TATCAACAGAAACGCACCGAG
Microsatellite	AthCTRI - Reverse	CCACTTGTTTCTCTCTCTAG
Microsatellite	AthSO392 - Forward	GTTGATCGCAGCTTGATAAGC
Microsatellite	AthSO392 - Reverse	TTGGAGTTAGACACGGATCTG

Table 6: 2c genomic DNA content for seven samples of *Arabis georgiana* analyzed using flow cytometry.

Sample ID	Genome Size	Standard
Black's Bluff 1	2.78 pg/2c	External
Black's Bluff 2	2.62 pg/2c	Internal
Black's Bluff 3	2.68 pg/2c	External
Black's Bluff 4	2.68 pg/2c	External
Black's Bluff 5	2.68. pg/2c	Internal
Fort Gaines 2	2.63. pg/2c	Internal
Fort Gaines 4	2.69. pg/2c	Internal
Mean	**2.69 (± 0.0193 SE) pg/2c**	

Table 7: *Arabis georgiana* populations analyzed for sequence polymorphism in the *trnL* intron of the cpDNA genome.

Population	# of Samples
Black's Bluff, Georgia	7
Fort Benning, Alabama	3
Fort Benning, Georgia	4
Goat Rock, Georgia	15
Resaca, Georgia	10
Whitmore's Bluff, Georgia	9
Total	**48**

Table 8: Preliminary screening of 15 samples of *Arabis georgiana* for microsatellite variation across 5 loci (DnB123a, DnA214, DnB220, AthCTRI, and AthS0392) (Ecogenics, GmbH, Zurich-Schlieren, Switzerland). Numbers below each locus indicate the base pair size of each respective microsatellite fragment.

Sample	DnB123a	DnA214	DnB220	AthCTRI	AthSO392
DB1	150, 175, 192	195, 196, 197, 198	205, 211	141	161
DB2	150, 175, 192	195, 196, 197, 198	205, 211	141	161
FBA60	150, 173, 192	0	205	141	161
FBA63	0	0	0	0	0
FBG12	150, 173, 192	196	202, 205	141	161
FBG49	150, 173, 192	195	202, 205	141	161
GRM12	150, 173	0	202, 205	141	161
GRM50	150, 173, 192	195, 196, 197, 198	202, 205	141	161
GRR40	150, 172, 192	195, 196, 197, 198	202, 205	141	161
GRS12	150, 172, 192	195, 196, 197	202, 205	141	161
GRS14	150, 172, 192	195, 196, 197, 198	202, 205	141	161
PB1	150, 175, 192	195, 196, 197, 198	205, 211	141	161
PB2	150, 175, 192	195, 196, 197, 198	205, 211	141	161
R1	150, 175, 192	195, 196, 197	205, 209	141	161
R10	150, 175, 192	195, 196, 197, 198	205, 209	141	161
Size Range*	**132, 154-157, 174**	**176-180**	**184-193**	**123**	**143**
No. of alleles	**1, 3, 1**	**4**	**4**	**1**	**1**

Table 9: Number of *Arabis georgiana* samples per population that positively amplified for both DnA224 and DnB220 microsatellite loci.

Population Name	**# Used for Microsatellite Analysis**
Black's Bluff, GA.	10
Whitmore's Bluff, GA	7
Oostanaula Bluffs, GA	8
Goat Rock, GA	10
Fort Benning, GA	6
Fort Benning, AL	10
Fort Gaines, GA	15
Pratt's Ferry, AL	10
Prarie Bluff, AL	12
Durant's Bend, AL	13
Total	**101**

Table 10: Comparison of 2005 and 2010 census data for all remaining populations of *Arabis georgiana* endemic to Alabama and Georgia.

Population	State	County	# of Plants (2005)	# of Plants (2010)
Black's Bluff*	GA	Floyd	3	81
Oostanaula Bluffs	GA	Floyd	32	42
Whitmore's Bluff	GA	Gordon	50	12
Ft. Gaines	GA	Clay	142	112
Goat Rock	GA	Harris / Muscogee	999	2320
Fort Benning	GA	Chattahoochee	880	886
Ft. Benning	AL	Russell	162	598
Limestone Park	AL	Bibb	unknown	50
Murphy Rd Bridge Bluff	AL	Bibb	unknown	18
Brown's Dam Glades	AL	Bibb	unknown	93
Fern Glades	AL	Bibb	unknown	81
Sixmile Creek	AL	Bibb	unknown	59
Creekside Glades	AL	Bibb	unknown	60
Little Schultz Creek	AL	Bibb	unknown	29
Pratt's Ferry	AL	Bibb	unknown	307
NW Pratt's Ferry	AL	Bibb	unknown	unknown
Durant's Bend	AL	Dallas	unknown	unknown
Portland Landing	AL	Dallas	unknown	42
Ft. Toulouse Natl. Historic Park	AL	Elmore	unknown	48
Marshall's Bluff	AL	Monroe	unknown	344
Fort Tombecbee	AL	Sumter	unknown	4
Prairie Bluff	AL	Wilcox	unknown	551
Range Total			**2103**	**5737**

* Represents an increase due to augmentation from *ex situ* reared plants

Table 11: Values for 2c DNA content of *Arabis georgiana* and other members of Brassicaceae with a base chromosome number of 8. *Arabis georgiana* is given the preliminary assignment as an octoploid species.

Species	Base Chromosome #	Ploidy	Nuclear DNA content (pg per 2C)
Arabis georgiana	8 (unverified)	8x (preliminary)	2.7 (±0.019)
Arabis pycnocarpa	8	4x 8x	unknown unknown
Arabidopsis lyrata	8	2x hybrid 4x	0.5 (±0.1) 0.8 0.9 - 1.1 (±0.1)
Draba altaica	8	2x	0.55 (±0.044)
Draba fladnizensis	8	2x	0.55 (±0.06)
Draba lactea	8	4x 6x	1.16 (±0.068) 1.89 (±0.217)
Draba nivalis	8	2x	0.6 (±0.037)

*Methods for 2C content based on work by Kron, 2012; Koch et al., 2010; Warwick and Al-Shehbaz, 2006; Johnston et al., 2005; Koch 2000, Dart et al., 2004; and Grundt, 2005.

Table 12: Identity matrix for *trnL* intron sequence polymorphism showing the percentage of bases that are identical between samples.

Sequence Identity	*A. georgiana* GU181971	*A. georgiana* GRL10	*A. georgiana* R04	*A. pycnocarpa* FJ188198	*A. pycnocarpa* FJ188213	*A. patens* FJ1288264	*A. patens* FJ188152	*A. blepharophylla* FJ188288
A.georgiana GU181971		100%	100%	100%	100%	99%	99%	99%
A. georgiana GRL10	100%		100%	100%	100%	99%	99%	99%
A. georgiana R04	100%	100%		100%	100%	99%	99%	99%
A. pycnocarpa FJ188198	100%	100%	100%		100%	99%	99%	99%
A. pycnocarpa FJ188213	100%	100%	100%	100%		99%	99%	99%
A. patens FJ1288264	99%	99%	99%	99%	99%		100%	99%
A. patens FJ188152	99%	99%	99%	99%	99%	100%		99%
A. blepharophylla FJ188288	99%	99%	99%	99%	99%	99%	99%	

Table 13: Allele frequencies for the microsatellite locus DnB220 across the three genetic clusters (K=3) of *Arabis georgiana* found across the species range. NG = North Georgia, SG = South Georgia and AL = Alabama. Allele numbers indicate the size (bp) of each microsatellite fragment.

Allele #	NG	SG	AL
184	0.000	0.366	0.000
186	0.500	0.634	0.500
190	0.500	0.000	0.000
192	0.000	0.000	0.500

Table 14: Unbiased Nei genetic identity for a) K = 4 with Fort Benning, (FBA) as a separate population and b) K = 3 with North Georgia (NG), South Georgia (SG) and Alabama (AL) comprising three genetic clusters.

a)

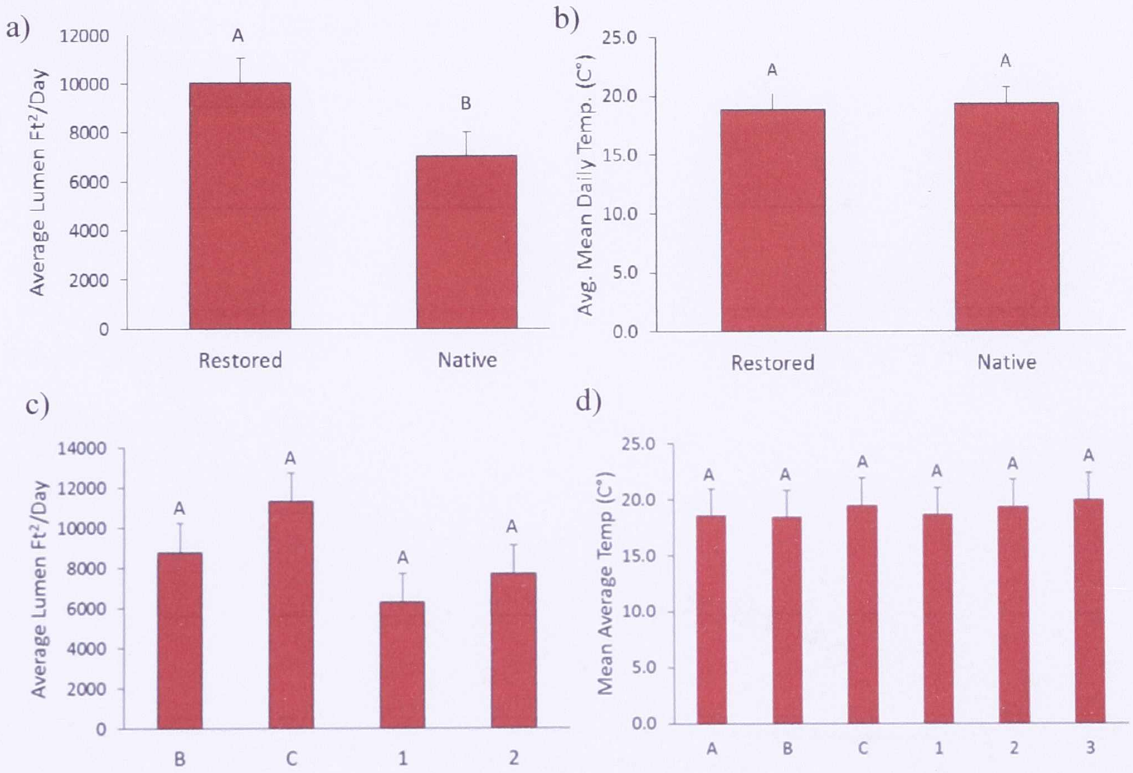
Pop1	Pop2	Unei GD
NG	SG	0.62
NG	FBA	0.34
SG	FBA	0.28
NG	AL	0.68
SG	AL	0.62
FBA	AL	0.34

b)

Pop1	Pop2	Unei GD
NG	SG	0.47
NG	AL	0.68
SG	AL	0.48

APPENDIX

Appendix A: Post-hoc analysis of variation between native and restored plots for environmental factors: a) light received per day in lumen feet ^{2} for restored and native plot types ($F_{1,46} = 4.61$, $p < 0.05$); b) mean daily temperature between for restored and native plot types ($F_{1,46} = 4.62$, $p > 0.05$); c) average amount of light received per day for individual plots in lumen feet^2 ($F_{3,44} = 2.24$, $p > 0.05$); d) mean daily temperature ($F_{3,44} = 0.05$, $p > 0.05$).



Appendix B: Report on genome size estimate for *Arabis georgiana* prepared Apr 24, 2012 by Paul Kron, Department of Integrative Biology, University of Guelph (Brian Husband lab)

Genome size estimate

The genome size estimate was based on 8 individuals, each tested only once (some plants were tested twice, once with internal and once with external standardization; in these cases, only the results with internal standardization are given below). The estimates for each individual are the following:

Black's Bluff 1	2.78 pg/2c (external standard)
Black's Bluff 2	2.62 pg/2c (**internal** standard)
Black's Bluff 3	2.68 pg/2c (**internal** standard)
Black's Bluff 4	2.68 pg/2c (external standard)
Black's Bluff 5	2.68. pg/2c (**internal** standard)
Fort Gaines 2	2.63. pg/2c (**internal** standard)
Fort Gaines 4	2.69. pg/2c (**internal** standard)

The genome size estimate across all individuals (mean of above 7 means) is:
2.69 (±0.0193 SE) pg/2c.

The original data was previously provided in a separate file.

Quality issues: Best practice standards for genome size studies state that each individual should be replicated 3 to 4 times, and at least 3 (4) plants should be used (eg. Greilhuber et al., 2007). It was not possible to replicate samples in this study because too little was available and what was available had deteriorated to some extent.

Our goal is to have peak CV's <5% (preferably <3%) and nuclei numbers per peak >1,300 (following standard recommendations for plants, such as Greilhuber et al., 2007 and Dolezel et al., 2007). In this case, all CV's were less than 4.2%. Radish nuclei numbers exceed 1000 in all cases and exceeded 1300 in more than half. Arabis nuclei exceeded 1,300 in all but 1 case (BB2 had only 185 in the March 2 sample but was also run March 6).

Endopolyploidy: *Arabis georgiana* is somewhat endopolyploid, with distinct 2c and 4c peaks in some samples. Endopolyploidy is a condition that appears to be widespread in the Brassicaceae.

Inhibition effects: We did not test for inhibition effects (*sensu* Price et al., 2000).

Methods

Tissue: Fresh leaves were sent by courier to the University of Guelph, in a cooler with an ice pack. The tissue arrived in relatively poor condition. The leaves were kept cool and

moist until testing (2 to 6 days after collection). For one sample (Fort Gaines 04) some stem and root was included because of very limited leaf tissue.

Sample preparation: The sample preparation method is based on the original method of Galbraith *et al.,* (1983); see also Dolezel et al. (2007). Each leaf was chopped with a new razor blade in a petri dish with 0.6 mL of ice-cold extraction buffer, along with leaf tissue from a DNA content standard (*Raphanus sativus*). For both the standard and the test plant, approximately 25mm^2 of tissue was used (up to twice this in some cases when available). After chopping for approximately 15s, the sample was filtered through a 30µm Partec Celltrics filter. The nuclei were allowed to stain in this buffer for 20-60 minutes before testing.

Extraction buffer: The extraction buffer contained 100µg/ml propidium iodide (PI) and 50µg/ml RNAse.. For this study, we used LB01 buffer (Dolezel et al., 1989). We did not do initial testing of other buffers because the amount of tissue available was to limited and the quality of the output was good in LB01 for samples with sufficient healthy tissue.

DNA content standard: We used *Raphanus sativus* L. 'Saxa'. The published DNA content value for this species and variety is 1.11 pg/2c (Dolezel et al. 1992).

Sample testing: The samples were run on a BD FACSCalibur flow cytometer (BD Biosciences, San José, USA). Samples were run on low for long enough to acquire at least 1,300 nuclei per G1 peak (following quality recommendations of Greilhuber et al., 2007), although this goal was not achieved in all cases. Samples were run up to 12 minutes to achieve these counts. We used the FL2 detector (585/42nm) to measure PI fluorescence, and used the integrated fluorescence (fluorescence area) as the parameter of interest.

Analysis: The FL2-Area histograms were analyzed using ModFit LT for Mac software (Vers. 3.3.11, Verity Software House, Inc., 2011. www.vsh.com). This software was used to measure the peak means, coefficients of variation (CV's) and nuclei number per peak.

The DNA content of the nuclei from the test plant were calculated as:
(peak mean of test plant)/(peak mean of standard) x (DNA content of standard). In this case, the DNA content of the standard was 1.11 pg/2c.

Inhibition testing: Best practice recommendations include testing for inhibition effect (in which the test species suppresses fluorescence in the standard and biases genome size estimates; Price et al. 2000). We were unable to do this because of the limits on tissue and time.

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Appendix C: rbcL sequences for *Arabis georgiana* and *Boechera laevigata* from populations where species co-occur (Black's Bluff, Whitmore's Bluff and Oostanaula Bluffs) and populations where only *Arabis georgiana* is known to occur (Fort Benning and Goat Rock).

***Arabis georgiana* (n=7)**

BlacksBluff1_Arabis_georgiana
TTTTGAAACGGTCTCTCCAACGCATAAATGGTNGGGAGTTCACATTCTCA
TCATCTWTGGTAAAATCAAGTCCACCACGTAGACATTCATAAACTGCTCT
ACCATAGTTCTTCGCGGATMMCCYCAATTTAGGTTTAATAGTACATCCTA
ATAGGGGACGCCCATACTTGTTCAATTTATCTCTTTCAACTTGGATACCA
TGAGGTGGTCCCTGGAAAGTTTTAGTATAAGCAGGAGGGATTCGCAGATC
CTCTAGACGTAGAGCCGCCAGGGCTTTGAACCCAAATACATTACCCACAA
TCGAGGTAAACATGTTAGTAACCGAACCTTCTTCAAAAAGGTCTAAGGGG
TAAGCTACATATGCAATAAATTGAGTTTCTTCTCCTGGAACGGGTTCAAT
GTGGTAGCATCGTCCTTTGTAACGATCAAGGCTGGTAAGCCCATCGGTCC
ACACAGTTGTCCATGTACCAGTAGAAGATTCAGCAGCTACCGCAGCCCCT
GCTTCTTCAGGTGGAACTCCGGGTTGAGGAGTTACTCRRAAKGCTGCCAA
GATATCAGTATCCTTGGTTTCATATTCAGGAGTATAATAAGTCAATTTAT
ATTCTTTAACMCCAGCTKWSAATCCAACACTTGCTTTAGTCTCTGTTTGT
GGTGACATAAAA

BlacksBluff2_Arabis_georgiana
NAAATGGTTGGGAGTTCACATTCTCATCATCTTTGGTAAANTCAAGTCCA
CCACGTAGACATTCATAAACTGCTCTACCATAGTTCTTCGCGGATAACCC
CAATTTAGGTTTAATAGTACATCCTAATAGGGGACGCCCATACTTGTTCA
ATTTATCTCTTTCAACTTGGATACCATGAGGTGGTCCCTGGAAAGTTTTA
GTATAAGCAGGAGGGATTCGCAGATCCTCTAGACGTAGAGCCGCCAGGGC
TTTGAACCCAAATACATTACCCACAATCGAGGTAAACATGTTAGTAACCG
AACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATATGCAATAAATTGA
GTTTCTTCTCCTGGAACGGGTTCAATGTGGTAGCATCGTCCTTTGTAACG
ATCAAGGCTGGTAAGCCCATCGGTCCACACAGTTGTCCATGTACCAGTAG
AAGATTCAGCAGCTACCGCAGCCCCTGCTTCTTCAGGTGGAACTCCGGGT
TGAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCCTTGGTTTCATA
TTCAGGAGTATAATAAGTCAATTTATATTCTTAAC

FtBennAL_Arabis_georgiana
AAATGGTTGGGAGTTCACATTCTCATCATCTWTGGTAAAATCAAGTCCAC
CACGTAGAACATTCATAAACTGGCTCTACCATAGTTCTTCGCGGATAACC
CCAATTTAGGTTTAATAGTACATCCTAATAGGGGACGCCCATACTTGTTC
AATTTATCTCTTTCAACTTGGATACCATGAGGTGGTCCCTGGAAAGTTTT
AGTATAAGCAGGAGGGATTCGCAGATCCTCTAGACGTAGAGCCGCCAGGG
CTTTGAACCCAAATACATTACCCACAATCGAGGTAAACATGTTAGTAACC
GAACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATATGCAATAAATTG
AGTTTCTTCTCCTGGAACGGGTTCAATGTGGTAGCATCGTCCTTTGTAAC
GATCAAGGCTGGTAAGCCCATCGGTCCACACAGTTGTCCATGTACCAGTA
GAAGATTCAGCAGCTACCGCAGCCCCTGCTTCTTCAGGTGGAACTCCGGG
TTGAGGAGTTACTCGGAAKGCTGCCAAGATATCAGTATCCTTGGTTTCAT
ATTCAGGAGTATAATAAGTCAATTTATATTCTTTAACACCAGCTTTGAAT
CCAACACTT

FtBennAL2_Arabis_georgiana
AAATGGTTGGGAGTTCACATTCTCATCATCTTTGGTAAAATCAAGTCCAC
CACGTAGACATTCATAAACTGCTCTACCATAGTTCTTCGCGGATAACCCC
AATTTAGGTTTAATAGTACATCCTAATAGGGGACGCCCATACTTGTTCAA
TTTATCTCTTTCAACTTGGATACCATGAGGTGGTCCCTGGAAAGTTTTAG
TATAAGCAGGAGGGATTCGCAGATCCTCTAGACGTAGAGCCGCCAGGGCT
TTGAACCCAAATACATTACCCACAATCGAGGTAAACATGTTAGTAACCGA
ACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATATGCAATAAATTGAG
TTTCTTCTCCTGGAACGGGTTCAATGTGGTAGCATCGTCCTTTGTAACGA
TCAAGGCTGGTAAGCCCATCGGTCCACACAGTTGTCCATGTACCAGTAGA
AGATTCAGCAGCTACCGCAGCCCCTGCTTCTTCAGGTGGAACTCCGGGTT
GAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCCTTGGTTTCATAT
TCAGGAGTATAATAAGTCAATTTATATTCTTTAACACCAGCTTTGAATCC
AACACTT

GoatRockMiddle_Arabis_georgiana
AAATGGTTGGGAGTTCACATTCTCMTCATCTTTGGTAAAATCAAGTCCAC
CACGTAGACATTCATAAACTGCTCTACCATAGTTCTTCGCGGATAACCCC
AATTTAGGTTTAATAGTACATCCTAATAGGGGACGCCCATACTTGTTCAA
TTTATCTCTTTCAACTTGGATACCATGAGGTGGTCCCTGGAAAGTTTTAG
TATAAGCAGGAGGGATTCGCAGATCCTCTAGACGTAGAGCCGCCAGGGCT
TTGAACCCAAATACATTACCCACAATCGAGGTAAACATGTTAGTAACCGA
ACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATATGCAATAAATTGAG
TTTCTTCTCCTGGAACGGGTTCAATGTGGTAGCATCGTCCTTTGTAACGA
TCAAGGCTGGTAAGCCCATCGGTCCACACAGTTGTCCATGTACCAGTAGA
AGATTCAGCAGCTACCGCAGCCCCTGCTTCTTCAGGTGGAACTCCGGGTT
GAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCCTTGGTTTCATAT
TCAGGAGTATAATAAGTCAATTTATATTCTTTAACACCAGCTTTGAATCC
AACACTT

Oostanaula Bluffs_Arabis_georgiana
AAATGGTTGGGAGTTCACATTCTCATCATCTTTGGTAAAATCAAGTCCAC
CACGTAGACATTCATAAACTGCTCTACCATAGTTCTTCGCGGATAACCCC
AATTTAGGTTTAATAGTACATCCTAATAGGGGACGCCCATACTTGTTCAA
TTTATCTCTTTCAACTTGGATACCATGAGGTGGTCCCTGGAAAGTTTTAG
TATAAGCAGGAGGGATTCGCAGATCCTCTAGACGTAGAGCCGCCAGGGCT
TTGAACCCAAATACATTACCCACAATCGAGGTAAACATGTTAGTAACCGA
ACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATATGCAATAAATTGAG
TTTCTTCTCCTGGAACGGGTTCAATGTGGTAGCATCGTCCTTTGTAACGA
TCAAGGCTGGTAAGCCCATCGGTCCACACAGTTGTCCATGTACCAGTAGA
AGATTCAGCAGCTACCGCAGCCCCTGCTTCTTCAGGTGGAACTCCGGGTT
GAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCCTTGGTTTCATAT
TCAGGAGTATAATAAGTCAATTTATATTCTTTAACACCAGCTTTGAATCC
AACACTT

WhitmoresBluff_Arabis_georgiana
AAGTGTTGGATTCAAAGCTGGTGGTWAAGAATATAAATTGACTTATTATA
CTCCTGAATATGAAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTA
ACTCCTCAACCCGGAGTTCCACCTGAAGAAGCAGGGGCTGCGGTAGCTGC
TGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCA
GCCTTGATCGTTACAAAGGACGATGCTACCACATTGAACCCGTTCCAGGA
GAAGAAACTCAATTTATTGCATATGTAGCTTACCCCTTAGACCTTTTTGA
AGAAGGTTCGGTTACTAACATGTTTACCTCGATTGTGGGTAATGTATTTG
GGTTCAAAGCCCTGGCGGCTCTACGTCTAGAGGATCTGCGAATCCCTCCT
GCTTATACTAAAACTTTCCAGGGACCACCTCATGGTATCCAAGTTGAAAG
AGATAAATTGAACAAGTATGGGCGTCCCCTATTAGGATGTACTATTAAAC
CTAAATTGGGGTTATCCGCGAAGAACTATGGTAGAGCAGTTTATGAATGT
CTACGTGGTGGACTTGATTTTACCAAAGATGATGAGAATGTGAACTCCCA
ACCATTT

***Boechera laevigata* (n=2)**

Oostanaula Bluffs_Boechera_laevigata1
NNANNNANNNAAAAGTGTTGGATTCAAAGCTGGTGKTWAAGAGTATAAAT
TGACTTATTATACTCCTGAATATGAAACCAAGGATACTGATATCTTGGCA
GCATTCCGAGTAACTCCTCAACCCGGAGTTCCACCTGAAGAAGCAGGGGC
TGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCG
ATGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAG
CCCGTTCCAGGAGAAGAAACTCAATTTATTGCGTATGTAGCTTACCCCTT
AGACCTTTTTGAAGAAGGTTCGGTTACTAACATGTTTACCTCGATTGTGG
GTAATGTATTTGGGTTCAAAGCCCTGGCTGCTCTACGTCTAGAGGATCTG
CGAATCCCTCCTGCTTATACTAAAACTTTCCAGGGACCACCTCATGGTAT
CCAAGTTGAAAGAGATAAATTGAACAAGTATGGACGTCCCCTATTAGGAT
GTACTATTAAACCAAAATTGGGGTTATANCGNGAAGAAACTACGGTAGAG
CAGTTTATGAATGTCTACGTGGTGGACTTGATTTWMCCAAAGATGATGAG
AATGTGAACTCCCAACCATTT

WhitmoresBluff_Boechera_laevigata
AAATGGTTGGGAGTTCACATTCTCNTCATCTTTGGKWAAATCAAGTCCAC
CACGTAGACATTCATAAACTGCTCTACCGTAGTTCTTCGCGGATAACCCC
AATTTTGGTTTAATAGTACATCCTAATAGGGGACGTCCATACTTGTTCAA
TTTATCTCTTTCAACTTGGATACCATGAGGTGGTCCCTGGAAAGTTTTAG
TATAAGCAGGAGGGATTCGCAGATCCTCTAGACGTAGAGCAGCCAGGGCT
TTGAACCCAAATACATTACCCACAATCGAGGTAAACATGTTAGTAACCGA
ACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATACGCAATAAATTGAG
TTTCTTCTCCTGGAACGGGCTCGATGTGGTAGCATCGTCCTTTGTAACGA
TCAAGGCTGGTAAGCCCATCGGTCCACACAGTTGTCCATGTACCAGTAGA
AGATTCAGCAGCTACCGCAGCCCCTGCTTCTTCAGGTGGAACTCCGGGTT
GAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCCTTGGTTTCATAT
TCAGGAGTATAATAAGTCAATTTATACTCTTTAACACCAGCTTTGAATCC
AACACTT

Appendix D: trnL sequences of samples representing six separate populations of *Arabis georgiana.*

BB02 Black's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

BB05 Black's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

BB06 Black's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

BB07 Black's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

BB08 Black's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

BB09 Black's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

BB10 Black's Bluff
GACTTAATTGGATTGAGCCTTGGTATKGRAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

FBA49 Fort Benning, Alabama
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

FBA55 Fort Benning, Alabama
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

FBA78 Fort Benning, Alabama
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

FBG03 Fort Benning, Georgia
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

FBG12 Fort Benning, Georgia
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTRTAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

FBG18 Fort Benning, Georgia
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

FBG19 Fort Benning, Georgia
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

FBG21 Fort Benning, Georgia
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

GRL10 Goat Rock
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

GRL12 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRL20 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRL32 Goat Rock
CGCTACGGACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGA
TAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAG
CCAAATCCTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGA
GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAC
TACCTTGTATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGT
GGAACTTATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTC
AATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTT
AAAAT

GRM12 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRM13 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRM24 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRM25 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRR01 Goat Rock
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

GRR31 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRS09 Goat Rock
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

GRS10 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRS11 Goat Rock
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

GRS12 Goat Rock
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

GRS14 Goat Rock
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R01 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R02 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R04 Oostanaula Bluffs
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

R06 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R07 Oostanaula Bluffs
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

R09 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R10 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R13 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

R14 Oostanaula Bluffs
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

R15 Oostanaula Bluffs
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

WB01 Whitmore's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

WB02 Whitmore's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

WB03 Whitmore's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

WB04 Whitmore's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

WB06 Whitmore's Bluff
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

WB07 Whitmore's Bluff
GACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTT
CAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATC
CTGGTTTACGCGAACAAACCTGCGTTTAAAAAGCGAGAAAAGAGGGATAG
GTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTG
TATTGATCAAAAGATTCACTTCATAGTCTGATAGATCCTTGGTGGAACTT
ATTAATTGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTG
ACAACAATGAAATTTATAGTAAGATGAAAATCCGTTGACTTTTAAAATCG
TGAGG

WB08 Whitmore's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

WB10 Whitmore's Bluff
CCTCACGATTTTAAAAGTCAACGGATTTTCATCTTACTATAAATTTCATT
GTTGTCAGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCAA
TTAATAAGTTCCACCAAGGATCTATCAGACTATGAAGTGAATCTTTTGAT
CAATACAAGGTAGTGAACTCCATTTGTTAGAACAGCTTCCATTGAGTCTC
TGCACCTATCCCTCTTTTCTCGCTTTTTAAACGCAGGTTTGTTCGCGTAA
ACCAGGATTTGGCTCAGGATTGCCCATTGTTAATTCCAGGGTTTCTCTGA
ATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATT
AAGTC

Appendix E: Microsatellite alleles for *Arabis georgiana* at locus DnA214 and DnB220 generated from FSA data files using Gene Mapper version 4.0 on default settings. Numbers shaded gray indicate no data was collected.

ID	DNA214		DNB220	
Ag_BB11	177	179	186	190
Ag_BB12	177	179	186	190
Ag_BB13	177	179	186	190
Ag_BB14	177	179	186	190
Ag_BB15	177	179	186	190
Ag_BB16	177	179	186	190
Ag_BB17	177	179	186	190
Ag_BB18	177	179	186	190
Ag_BB19	177	179	186	190
Ag_BB20	177	179	186	190
Ag_DB11	177	179	186	192
Ag_DB12	177	179	186	192
Ag_DB13	177	179	186	192
Ag_DB14	177	179	186	192
Ag_DB15	177	179	186	192
Ag_DB16	177	179	186	192
Ag_DB3	177	179	186	192
Ag_DB4	177	179	186	192
Ag_DB5	177	179	186	192
Ag_DB6	177	179	186	192
Ag_DB7	177	179	186	192
Ag_DB8	177	179	186	192
Ag_DB9	177	179	186	192
Ag_FBA25	177	179	186	186
Ag_FBA31	177	179	186	186
Ag_FBA48	177	179	186	186
Ag_FBA49	177	179	186	186
Ag_FBA55	177	179	186	186
Ag_FBA56	177	179	186	186
Ag_FBA57	177	179	186	186
Ag_FBA61	177	179	186	186
Ag_FBA62	177	179	186	186
Ag_FBG15	177	179	184	186
Ag_FBG24	177	179	184	186
Ag_FBG28	177	179	184	186

Ag_FBG29	177	179	186	186
Ag_FBG34	177	179	184	186
Ag_FBG35	177	179	184	186
Ag_FBG40	177	179	184	186
Ag_FBG47	0	0	0	0
Ag_FtG10	177	179	184	186
Ag_FtG13	177	179	184	186
Ag_FtG14	177	179	184	186
Ag_FtG15	177	179	184	186
Ag_FtG16	177	179	184	186
Ag_FtG27	177	179	184	186
Ag_FtG3	177	179	184	186
Ag_FtG30	177	179	184	186
Ag_FtG31	177	179	186	186
Ag_FtG4	177	179	184	186
Ag_FtG5	177	179	184	186
Ag_FtG6	177	179	184	186
Ag_FtG7	177	179	184	186
Ag_FtG8	177	179	184	186
Ag_FtG9	177	179	184	186
Ag_GRL10	177	179	184	186
Ag_GRL34	177	179	184	186
Ag_GRL40	177	179	184	186
Ag_GRM12	177	179	184	186
Ag_GRM24	177	179	184	186
Ag_GRR19	177	179	184	186
Ag_GRR36	177	179	184	186
Ag_GRS10	177	179	184	186
Ag_GRS11	177	179	184	186
Ag_GRS6	177	179	184	186
Ag_PB10	177	179	0	0
Ag_PB11	177	179	186	192
Ag_PB12	177	179	186	192
Ag_PB13	177	179	186	192
Ag_PB14	177	179	186	192
Ag_PB15	177	179	186	192
Ag_PB3	177	179	186	192
Ag_PB4	177	179	186	192
Ag_PB5	177	179	186	192

Ag_PB6	177	179	186	192
Ag_PB7	177	179	186	192
Ag_PB8	177	179	186	192
Ag_PB9	177	179	186	192
Ag_PF1	177	179	186	192
Ag_PF10	177	179	186	192
Ag_PF11	177	179	186	192
Ag_PF12	177	179	186	192
Ag_PF2	177	179	186	192
Ag_PF3	177	179	186	192
Ag_PF4	177	179	186	192
Ag_PF6	177	179	186	192
Ag_PF7	177	179	186	192
Ag_PF9	177	179	186	192
Ag_Res1	177	179	186	190
Ag_Res11	177	179	186	190
Ag_Res12	177	179	186	190
Ag_Res13	177	179	186	190
Ag_Res14	177	179	186	190
Ag_Res3	177	179	186	190
Ag_Res5	177	179	186	190
Ag_Res8	177	179	186	190
Ag_WB1	177	179	186	190
Ag_WB10	177	179	186	190
Ag_WB2	177	179	186	190
Ag_WB4	177	179	186	190
Ag_WB5	177	179	186	190
Ag_WB6	177	179	186	190
Ag_WB8	177	179	186	190